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EXPERIMENTAL YAWS IN PHILIPPINE MONKEYS AND A CRITICAL CONSIDERATION OF OUR KNOWLEDGE CONCERNING FRAMBŒSIA TROPICA IN THE LIGHT OF RECENT EXPERIMENTAL EVIDENCE

By OTTO SCHÖBL

Chief, Division of Biology and of Serum Laboratory
Bureau of Science, Manila

THIRTY PLATES AND ONE TEXT FIGURE

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INTRODUCTION

The disease known as frambœsia tropica, or yaws, presents no practical problem to the practitioner. The etiology of the disease has been established and it is one of the easiest, if not the easiest, of the systemic diseases of man to cure. Therefore, it would at first sight seem superfluous to conduct experiments in quest of further knowledge of a disease the basic facts concerning which appear to be so well established. However, as will be seen, there are several points of importance as well as of interest that require elucidation. The literature on experimental yaws is very limited in comparison with that concerning experimental syphilis. We may safely state that, in this and many other tropical countries, there are far more people affected by yaws than by syphilis. Castellani(3) said in 1906 that "numerous experiments are necessary;" Ashburn and Craig(1) remarked in 1907, "The literature relating to the production of frambœsia is monkeys * * * is very limited;" and Sellards and Goodpasture(29) in 1923 complain that "the experimental data are extremely meager" and "the evidence of tertiary manifestations rests chiefly upon clinical grounds and not upon any exact demonstration in the laboratory."

From the epidemiologic viewpoint the mode of transmission of the disease among humans is not very clear. The question of immunity is unsettled. Moreover, the question of the relation of yaws to syphilis still awaits an answer.

DISCUSSION OF THE PROBLEM

The problem as it presents itself for experimental study on animals is best made evident by a critical analysis of the experiments already performed by various authors who, in particular, have inquired into the problems of cross immunity and the question of the identity of frambœsia and syphilis. Shortly after the discovery by Castellani of the cause of yaws, several groups of investigators undertook the experimental study of the disease. Inspection of the available literature makes it apparent that the outstanding workers in this field are considered to be Neisser et al.,(20) in Java; Castellani,(5) in Ceylon; Levaditi et al.,(13) in France; and Ashburn and Craig,(1) in the Philippines. These investigations, as already stated, were begun shortly after the discovery by Castellani of *Treponema pertenue;* that is, in 1906 and 1907. Nothwithstanding the lapse of two decades, this work still is being quoted in textbooks and other

publications as though the problem of immunity and, consequently, the question of the nonidentity of yaws and syphilis had been settled beyond peradventure at that early period. It soon becomes evident to anyone who undertakes a critical analysis of these early experiments that these several groups of students arrived at conclusions that may be mildly characterized as contradictory. That is, in some instances their findings are in agreement and in others they are in disagreement with experimental observations made on yaws as the disease occurs in man. The results of experimentation with yaws on the human subject are no less confusing; nor are they at all conclusive. With regard to experiments on immunity in yaws, it is at once evident that the question of identity or difference between yaws and syphilis was paramount in the minds of the investigators. In short, on reviewing the work of these authors, we discover that, while they developed a considerable volume of knowledge bearing on the subject in general, the conclusions they drew from their experiments on animals or on humans were at considerable divergence. One is led to the conclusion that the cause of the confusion is to be found in the experimental methods employed in some of the work. The number of experiments performed is too small to afford a scientific basis for the conclusions drawn. It is likewise seen that similarly definite conclusions were based on negative results, no consideration apparently having been given to the possibility that failure may have been due to the employment of faulty technical arrangement.

The investigations of Neisser(20) and his coworkers yield sufficient evidence to show that neither does syphilis immunize against yaws nor yaws against syphilis, when infection and superinfection take place under the conditions under which their experiments were conducted. However, the negative results attained in their efforts to produce superinfection with yaws in frambœsic animals require explanation, especially as their account fails to show that the viability and infectivity of the material they used for the superinfection of yaws monkeys were tested by inoculation into control animals. The same objection arises in connection with the work of Levaditi and Nattan-Larrier.(13) They were not able to produce yaws in syphilitic monkeys. Castellani,(6) however, proved that frambœsic animals can be infected with syphilis. Ashburn and Craig(1) are quoted as having concluded that Philippine monkeys are susceptible to yaws but not to syphilis. It seems quite evident from the foregoing, therefore, that these questions cannot be definitely

answered unless the course of the disease as it occurs in monkeys is thoroughly known, and unless a gradually developing immunity can be made apparent by definitely recognizable and reliable signs. Close attention must be paid to the character of the lesion in animals with a view of discovering in them such modification of the frambœsic manifestations as are known to exist in man. This is because convincing evidence of immunity cannot be considered to be afforded by the phrase "negative take," when at the same time it is clearly apparent that the material used in inoculation has not been tested in control animals and only one attempt at infecting the animal was made. Sellards and Goodpasture(29) crystallize the situation in the remark "Before concluding that yaws affords no protection whatever against syphilis, it would be desirable to demonstrate that some degree of immunity had been established to yaws itself before testing the resistance to syphilis." That these two diseases are closely related is admitted with greater or less frankness by all investigators, including the "Dualists." This is evident from such statements as "yaws is no more syphilis than leprosy is tuberculosis," and "the relation of the two diseases can be compared with the relation of tertiana to tropical malaria."

It would seem unnecessary to argue that this problem can be solved by other than experimental means; there are too many disturbing factors. Repeatedly, other origin or etiology has been assigned to lesions in man attributed to yaws. Even when it has been possible to demonstrate spirochætes in these lesions, thus fulfilling the first postulate of Koch, the question inevitably has arisen as to whether the spirochætes were *Treponema pallidum* or *T. pertenue*. Experimentation on man has thrown some light on certain phases of the problem; but, since spontaneous syphilis as well as yaws occurs in human beings, there always will remain a shadow of doubt as to the real etiology of some of the experimentally produced lesions. Moreover, the majority of yaws patients are ambulatory cases, and they present themselves with well-developed lesions, "primary," "secondary," or "tertiary" in type, as the case may be. The opportunity in the clinic to see the beginning of any type of frambœsia is exceedingly rare. With the introduction of salvarsan into the theraphy of yaws the possibility of following the cases throughout the entire course of the disease ceased to exist. After the first injection of the drug the skin manifestations heal with such rapidity that the patient deems it no longer necessary to report to the clinic. The same limitations fail in experiments on human volunteers. Sooner or

later the developing yaws reach such dimensions that it is not justifiable to delay treatment any longer and the entire process is terminated at a moment when the scientific interest really begins. Another factor that militates against clinical observations and experimentation on human beings with a view of deciding the etiology of protean lesions of yaws lies in the fact that the greatest variety of these lesions occurs naturally in heavily infested regions where practically everybody has, or has had, yaws. The choice of volunteers thereupon becomes difficult and sometimes impossible. In the face of such extensive infestations it is difficult to ignore the possibility that other skin lesions, having nothing whatever to do with frambœsia, may as well occur in a frambœsic patient as in one who is not suffering with yaws. In other words, the exclusion of nonspecific lesions by comparison under such conditions offers a difficult as well as a highly important problem. The difficulty of differential diagnosis of protean lesions of yaws in particular becomes far greater if one remembers the great similarity between certain manifestations of yaws and certain manifestations of syphilis. Even though spirochætes be found in a lesion the possibility of their being *Treponema pallidum* and not *T. pertenue* unfortunately exists. In 1912 Plehn(25) arrived at the conclusion that "one can therefore say with regard to differentiation of certain late frambœsic from late syphilitic lesion" [1] that "the possibility of further investigation in the clinical field lacks all grounds." In my judgment it would be beyond reason to demand the fulfillment of Koch's first postulate—the actual demonstration of *Treponema pertenue* in a lesion—as the absolute criterion for its determination as of frambœsic origin. If it be borne in mind that under natural conditions lower animals suffer from neither spontaneous syphilis nor spontaneous yaws, it will seem quite as apparent to others as it is to me that the production in these animals of lesions clinically and anatomically characteristic—in short, in main respects identical with those found in man—will afford as convincing proof of the etiology of the lesions as can possibly be produced at the present time. At all events, such a criterion meets the terms of the third postulate of Koch, which requires the experimental production of the disease.

An account of the inoculation experiments on animals lower than monkeys is not included in this short review of the history

[1] The translations of passages quoted from papers in languages other than English are mine.—O. S.

of experimental yaws in animals. The intratesticular inoculation of *Treponema pertenue,* like that of *T. pallidum,* is a valuable laboratory method to secure material in which the treponemas are free from bacterial contamination and to maintain them in that condition, as well as a preliminary step toward obtaining pure cultures of the organism. However, the possibilities of experimentally solving by this procedure some of the dark chapters in our knowledge of human frambœsia are very limited. The testicular tissue is neither the portal of entry nor even the favorite place of localization of *Treponema pertenue.* The experimental possibilities of this method have been practically exhausted by Nichols(21–23) and by Matsumoto, Ikegami, and Takasaki,(17) so far as frambœsia is concerned. The intratesticular inoculation of *Treponema pertenue,* being an artificially created condition, will not solve such problems in human yaws, as, for instance, the etiology of certain atypical or unique lesions encountered in yaws-infested countries, and it is questionable whether the problem of immunity to yaws in man can be solved by experimentation by means of intratesticular inoculation, as the testicle is positively known not to be affected in human frambœsia. It must be borne in mind that yaws, as far as the human is concerned, is primarily a skin disease, and in the majority of cases remains such, even though what may be called septicæmic distribution takes place. In that respect yaws resembles smallpox so closely that the analogy of those two diseases in their pathogenesis and immunity forces itself upon one's mind despite the fact that the two diseases differ from each other in many other respects. There is no more reason to consider yaws and syphilis identical in every respect just because the causative agents as far as morphology and biology are concerned are almost indistinguishable than there is reason for the assumption that the pathogenesis and immunity of tuberculosis and leprosy must be identical in every respect. As leprosy is primarily a skin disease, so is frambœsia, while tuberculosis affects preferentially the internal organs, an occurrence which is secondary in leprosy. The entire process and pathogenesis and, consequently, immunity in yaws is so complicated, and no doubt so changeable and different in its various stages, that the clinical course of yaws as it occurs in man must be duplicated in an animal before all the varieties and numerous stages in the pathogenesis of the frambœsic infection and therefore in the immunity can be discovered. The confusion and the contradictory and discordant results of the experiments by renowned

investigators of this wide and composite problem, both in animals and in humans, is the best proof of the complicated intricacy of the entire problem.

With the foregoing preamble the concrete consideration of the following problems, which have been attacked in these experiments, will now be given:

1. Does frambœsia tropica in monkeys run a course similar to that in man, or can it be induced to do so by special experimentation?

2. Does immunity to yaws exist in animals infected with yaws, and how does it present itself?

3. Is this immunity, if it exist, permanent, or does it last only during the stage of yaws infection?

Therefore, without having attempted any comparison or cross-experimentation between syphilis and yaws, I have endeavored to extend the knowledge of experimental yaws and its immunity in animals, at least to a point corresponding with the existing knowledge with respect to experimental syphilis and its immunity. Not until the pathogeny and immunity of yaws have been thoroughly investigated will it be possible to see the points in which these two diseases, syphilis and yaws, agree and those in which they differ. Furthermore, if lesions in man suspected to be of frambœsic origin can be duplicated in experimental animals no doubt can remain as to their identical etiology. In this respect the results of my experimentation here reported have surpassed all expectations.

TECHNICAL PART

The impression gained by early experimentators who attempted to transmit yaws to animals, that lower monkeys are not susceptible to this disease, was soon corrected. The possibility of successful transmission of yaws to Philippine monkeys was demonstrated by Ashburn and Craig,(1) and by Strong and Guerrero.[2] I was an eyewitness of successful inoculations made by E. L. Walker in 1912. As far as could be ascertained all of these workers, with the exception of Ashburn and Craig, made their inoculations exclusively into the eyebrows. Whether or not they were led by the statement of Halberstädter,(10) that anthropoid monkeys are inoculable in all parts of the body, but lower monkeys only in the eyebrows, I cannot say. If this statement of Halberstädter be true it would indicate that *Treponema*

[2] Oral communication.

pertenue not only has tissue-selective proclivities but also prefers skin tissue of a certain anatomical condition to skin tissue in which this condition is absent. In other words, it would indicate a local, what might be termed anatomical, tissue resistance. On the other hand, it may simply mean that the *Treponema* must be placed in a certain layer of the skin in order to obtain a foothold, and that this is more likely to happen in the skin of certain parts of the body than in that of others. The latter supposition is more likely to be true, since Ashburn and Craig produced a positive yaws lesion on the abdominal skin of a Philippine monkey by the pocket method. In my experiments inoculations were made in various parts of the monkey's body, not with the primary purpose to test the validity of the statement made by Halberstädter,(10) but rather to find the most convenient part of the body of the monkey in which transmission inoculation would invariably take. Another point necessary to decide in these experiments was to find the part of the body where the take and the frambœsic lesion would persist for the longest time or give the greatest yield of treponematous material, so that we should be able to maintain the strains of *Treponema pertenue* economically without having to waste many animals and without the necessity of constant vigilance for fear of losing the strains. Thus the above-mentioned contention of Halberstädter necessarily underwent a test.

TECHNIC OF INOCULATION

After several attempts to devise a suitable method for the transmission of yaws to monkeys, the following procedure was found most reliable and at the same time best for the purpose of clinically studying the development of the lesion.

The choice of material was dependent on the presence in sufficient numbers of *Treponema pertenue* in the lesion from which the inoculum was to be obtained. The oozing lymph from the lesion was therefore examined under the dark field, and when treponemas were found the lesion was considered suitable for the transmission experiment. The yaw material was scraped off with a scalpel and emulsified gently in not more than approximately equal parts of the scrapings and physiological salt solution at room temperature (28° C.). After a thorough emulsion had been made in salt solution another dark-field examination was made to enable us to judge approximately the amount to be injected according to the number of treponemas encountered in one loopful of the emulsion. This emulsion was injected into

the monkeys by means of a small, sterile hypodermic syringe. More than one monkey was injected with the amount of material and, when the injections were concluded, another dark-field examination was made of the remnant of the inoculum to make sure that the treponemas were evenly distributed in the inoculum and that all the injections performed contained approximately the same number of treponemas.

As yaws is primarily an infection of the skin, this part of the work was concerned mainly with inoculations into the skin; and, as the yaws lesion is predominantly located in the superficial layers of the skin and in man the treponemas are found in the same part of the skin in the largest numbers, the inoculations were made intradermally. By this method attempts were made to produce experimental yaws in the areas of tight and tough skin as well as in those portions of the body where the skin is loose and tender. No attempt was made in this study to produce or to study bone lesions. Injections were made into the following regions: The eyebrows, the nose, the breast and the mammæ, the tail, the periumbilical region, the scrotum, the prepuce, the palms of the hands, the lips, the nasal septum, the perianal region, and the labia majora. It may be of interest to note that repeated inoculations by injection or scarification into the nasal and vaginal mucous membrane persistently failed to take.

Intradermal injections were employed in the greatest number of experiments, in preference to scarification or the pocket method of inoculation. In this way, complications of the primary efflorescences arising through mechanical injuries caused by inoculation were largely avoided.

The needle was inserted subcutaneously some distance from the place at which the material actually was to be injected and the point of the needle was led up into the skin so as to perform the intradermal injection. In some experiments scarification and pocket insertion were used also, the particular object being to discover which of these methods would give the highest number of positive takes under otherwise equal conditions.

The results tabulated in Table 1 show that successful inoculations were obtained on the skin of the nose, tail, scalp, scrotum, and eyebrows, while the ears, mamilla, palms of the hands, labia majora, lips, and perianal region, as well as the chest and the abdomen, gave repeatedly negative results. These results naturally do not mean that the skin of those parts of the body where no lesions were produced by intradermal inoculation

or scarification are absolutely immune, and that an initial local lesion could not be produced at all. Metastatic lesions, or extension by continuity of local lesions to these anatomical regions, were observed from time to time. The results, however, show that the highest percentage of takes was obtained by intradermal inoculation in the scrotum and in the eyebrows. It should be further mentioned that the eyebrow lesion is more likely to run a short course and present the dry form of yaws, while the yaws lesion on the skin of the scrotum is very extensive, giving a large amount of material, and as a rule is of longer duration. Furthermore, the yaws lesion on the eyebrows is more exposed to accidental injury than is that on the scrotum.

TABLE 1.—*Showing the results of attempts at inoculation of yaws into the skin of various parts of the body in Philippine monkeys.*

Monkeys used.	Place of inoculation.	Result of inoculation.	
		Positive.	Negative.
4	Ears	0	4
5	Mamilla	0	5
4	Palms of hands	0	4
3	Labia majora and perianal region	0	3
15	Skin of nose	5	10
1	Tail	1	0
2	Chest and abdomen	0	2
1	Scalp	1	0
7	Scrotum	7	0
16	Eyebrows	14	2
3	Septum nasi	0	3

EXPERIMENTAL ANIMALS

The animals used in these experiments were common Philippine monkeys, usually termed *Cynomolgus philippinensis* Geoffroy. Most of these animals came from Laguna Province, though a few came from Bataan Province, both of which are near Manila. They were used at random; that is, old, vigorous, young and small, some recently captured, and some that had been kept in captivity for some time. No attempt was made at zoölogical identification. It was noticed that the age or physical condition of the animal bore no relation to susceptibility to yaws. As a matter of fact, some small and young animals gave faint lesions while vigorous and old animals, as it happened, gave the greatest variety of yaws lesions of the longest duration.

STRAINS OF TREPONEMA PERTENUE

All the strains of *Treponema pertenue* that were used in these experiments were obtained from patients in the Philippines. The strains were secured by direct inoculation from patient to monkey and maintained by inoculation from monkey to monkey. Text fig. 1, page 229, shows the successive passages. Altogether five strains were secured in this manner, but most of the experiments were conducted and the lesions described herein produced with the strain called by the patient's name "Cadangan." Besides this, several other strains were isolated in the lowlands; that is, in the vicinity of Manila. The mountain strains, called "Kiangan," for the locality where they were secured,(15) proved of low infectivity as far as monkeys are concerned. The lesions produced in monkeys on first inoculation with these strains were of very short duration and very mild, having healed suddenly in the early second stage of development of local yaws.

The actual number of treponemas necessary to reach the subepidermal layer is very small. We have seen typical frambœsic lesions develop following an inoculation of less than a half cubic centimeter of suspension of frambœsic material in which under dark-field illumination only one or two treponemas could be found in a search of from sixty to one hundred fields. The lesions developed in due time, provided the inoculum was placed intradermally. Indeed, typical lesions were produced by inoculation of yaws material in which a thorough search under the dark field failed to show any treponemas whatsoever.

SUSCEPTIBILITY OF PHILIPPINE MONKEYS TO YAWS

The susceptibility to yaws of the animals used by us is considerable and with a little care no difficulty is experienced in infecting Philippine monkeys with yaws; in the course of these investigations one hundred twenty Philippine monkeys have been successfully inoculated. Most of these animals were inoculated with one and the same strain of yaws from monkey to monkey. No difficulty was experienced in transmitting yaws to these animals for the first time directly from man. Of the seven attempts to transmit yaws from patients with florid yaws to monkeys only one failed. Five patients and seven animals were involved in these experiments of yaws transmission directly from man to animals.

CLINICAL PART

THE INITIAL EXPERIMENTAL FRAMBŒSIC LESION ON THE EYEBROWS OF PHILIPPINE MONKEYS

Immediately after the intradermal injection of 0.1 to 0.2 cubic centimeter of the inoculum into the eyebrows there is visible a flat, pale elevation which the next day is surrounded by slight œdema. This œdema disappears and there follows a period of lull. About three to five weeks after the injection there appears either a single papule, multiple acuminated papules, or a flat indurated papule, the first stage (Plate 1, fig. 1), which spreads gradually within the area of the eyebrow and presents a lobulated, indurated, raised, oval lesion with unbroken surface (Plate 1, fig. 2). This lesion sometimes measures 1 to 2 centimeters by 0.5 centimeter, is oval, and the coarse hair within the lesion of the eyebrows is preserved while the fine hair usually is missing by the time the lesion has reached these dimensions. Sooner or later there appear on the surface of the lesion erosions and rhagades, either traumatic or spontaneous. These erosions soon become covered with a rather soft, brittle crust of amber color. The coloration is imparted by the lymph which oozes from the fissures. The color of the crust covering the larger, traumatic erosions may be darker as a result of an admixture of blood with the lymph.

When the scabs are removed an oozing, slightly bleeding granulation tissue becomes visible. This lesion represents the second stage of the frambœsic process in monkeys and corresponds to the typical yaw in man.

Later there is noticeable a central flattening, so that the lesion assumes a circinated form, apparently healing in the center and progressing on the periphery. This constitutes the third stage, or "ringworm form." The spreading of the lesion in this stage is simultaneous along the entire margin of the original lesion. At the same time, it progresses as a linear lesion covered with thin, dry scabs. The extension of this lesion is much more rapid toward the forehead than it is toward the eyelid. It therefore assumes a semilunar form the convexity of which is toward the top of the skull. It then descends the bridge of the nose, producing a butterfly effect down the side of the nose where it appears as a linear lesion toward the outer canthus of the corresponding eye. As the lesion spreads it

becomes drier; there is less oozing and it becomes less elevated than it was in the beginning. The tissue reaction at this stage being very mild, the lesion gradually develops to a narrow line of dry scabs. These bear a closer resemblance to circinated psoriasis than they do to ringworm, to which they have frequently been likened. Before the lesion completely disappears it assumes the form of a narrow, flat, circinated line which undergoes a branlike desquamation and exhibits only slight hyperæmia at the base. Both the upper and the lower eyelids almost invariably escape involvement in this spreading framboesic lesion.

The progress of the lesion usually stops when it has reached the tip of the nose, the lower margin of the zygomatic bone below the eye, and the top of the skull above the eyebrow. In some instances, however, the lesion has entered the nostril and established itself on the internal surface of the ala nasi; that is, on the mucocutaneous border. It tends to persist there longer than on the face as dry, slightly adherent scabs without much reaction in the underlying tissue. It finally disappears completely.

Although the initial local yaw as a rule heals in the manner described above, two more stages in a local yaw are occasionally encountered. In as much as analogous late skin lesions of metastatic experimental yaws occur in animals, they are mentioned here as the fourth and the fifth stage. Not infrequently, after the yaws on the eyebrows and on the bridge of the nose have healed after having spread upward and downward beyond these regions, there remains, for a considerable time, on the eyebrows and the bridge of the nose, a dry desquamation on a rather thickened skin. This may be designated the fourth stage. After this, the skin on the bridge of the nose becomes shiny and, ultimately, irregular pigmented spots appear. These persist, forming the fifth stage (see Plate 17, fig. 1).

The description of the development of the framboesic lesion as given above is typical of the most extensive primary lesions observed arising, apparently, from a virulent strain of spirochæta in single-infected monkeys. We have, however, observed in monkeys injected either with poor inoculum or with strains of apparently low virulence (Kiangan mountain strains) that the lesion stopped and disappeared without having assumed the second stage described above. In other instances we have seen, the lesion has ceased to progress and healing has taken place before it has reached the upper limits on the skull and face as has been described. In other words, in the case of animals

inoculated for the first time, we have seen instances in which the progress of the lesion has been arrested, regression has taken place, and eventually all evidence of frambœsic infection has disappeared. This phenomenon, it should be noted, may occur at any stage in the development of the initial local lesion.

If the eyebrows on both sides be injected with the same material at the same time the resulting lesions, in either the second or the third stage, ultimately meet and become confluent without any apparent effect of one lesion upon the other, although one lesion may develop earlier than the other.

THE INITIAL EXPERIMENTAL FRAMBŒSIC LESION ON THE SCROTUM OF PHILIPPINE MONKEYS

In the previous section mention was made of the fact that the initial local lesion spreads from the eyebrows along the scalp down the bridge of the nose to the cheeks but persistently avoids the loose, soft skin of the lids. Many inoculations were made into the scrotum by the pocket method. That is, a piece of tissue with its adherent scabs was implanted in the subcutaneous tissue close to the cutaneous surface. The skin of the scrotum is similar to that of the eyelid in that it is thin and loose. However, the lesion so produced presented a somewhat different picture. A marked and extensive œdema appeared, which was followed by a deep induration at the place where the inoculum was implanted. Ultimately, there developed a superficial lesion that spread out irregularly. The surface was moist and was surrounded by an area of intact skin infiltrated by a rather hard œdema. In some places the margins were slightly undermined; in others they were flat. It is mainly this œdema of the surrounding skin that produces the ulcerlike appearance of the skin lesion. When the œdema is not very pronounced, the true character of the lesion is evident. It is then seen to be an elevated, granulomatous lesion, covered with brownish yellow scabs and surrounded by a slightly elevated reddish zone. The lesions spread without much healing in the center and involved the entire half of the scrotum and sometimes the base of the penis before they started to regress. The healing took place gradually, the regenerated skin dividing the large lesion into numerous small ones which gradually became covered with crust. The deep infiltration persisted.

When the inoculum was injected intradermally into the scrotum by means of a syringe the lesion proceeded similarly, except that the deep induration and the œdema were absent. It began

as a reddish papule, which gradually spread in the way just described (see Plate 2, fig. 1). The main characteristic of the primary lesion in soft skin, such as that of the scrotum, is that it is more extensive and does not show so much tendency to central healing as does the lesion on the eyebrows or on the nose.

THE INCUBATION PERIOD OF EXPERIMENTAL INITIAL LOCAL YAW IN PHILIPPINE MONKEYS

The incubation period in higher monkeys is given by Halberstädter as from twenty-two to fifty days, in gibbons from thirteen to fourteen days, and in *Macacus* from ninety-one to ninety-six days. Nichols gives the incubation period as twenty-four days; White and Tyzzer give it as sixteen days; Ashburn and Craig, approximately twenty days; Levaditi gives it as between twenty-four and fifty-two days. The initial lesions described above developed in our animals on an average in twenty-six days, the shortest incubation having been seventeen and the longest, fifty-two days.

THE DURATION OF EXPERIMENTAL INITIAL LOCAL YAW IN PHILIPPINE MONKEYS

The duration of the initial local yaw, experimentally produced in monkeys, is subject to great variations. Halberstädter(10) gives a duration of from three to four weeks in lower monkeys, while in the orang-utan the lesion has been observed to last more than three months. White and Tyzzer(32) noted the duration of the lesion for seven weeks, while Ashburn and Craig(1) reported persistence for a period of between ten and eighty-four days. In the case of two monkeys in our series inoculated with the "mountain strains," the duration was very short. The duration of a superciliary lesion in our experiments, provoked by the method given above and with inoculum containing a fair number of treponemas, was on the average eighteen days, while the scrotal lesion lasted twenty days. These figures show the duration of the actual initial yaw; that is, of the second stage.

LOCAL EXACERBATIONS OF INITIAL LOCAL EXPERIMENTAL YAW IN PHILIPPINE MONKEYS

The periodic exacerbations of apparently stationary or regressing local yaws lesions have been referred to in the literature as local "recidives."[3] In our experiments exacerbations were

[3] No period of latency intervenes between the original local yaws and the so-called "recidives." Consequently, the term exacerbations seems more appropriate.

noticed in a certain proportion of inoculated monkeys. The local exacerbations occurred not only in the eyebrows but also in the scrotum. A photograph of an unusual and extensive local exacerbation on the eyebrows and scrotum is shown in Plate 3. As a general rule, the local exacerbations are smaller in extent than is the original lesion. They always occur in the skin already affected, usually involving only a part of the area of the original lesion. As a rule, they are located on the margin of the old lesion and spread by continuity, running the same course as a primary yaw. They are rarely confluent, as in the case illustrated where the exacerbation resulted in a lesion much more intensive than the original experimental yaw. They are sometimes multiple when the healing skin has intersected the old lesion into two or more islands. Local exacerbations occurring in an original lesion that is healing in this manner are very misleading to those who are unacquainted with the past history of the lesion, and may be wrongly interpreted as multiple or metastatic lesions (see Plate 4). Local exacerbations never have been seen to occur in the normal skin surrounding the original lesion; therefore, no significance can be attached to them in the consideration of generalized yaws or even regional dissemination. They are, however, significant in the pathogeny of local yaws and characteristic of the nature of the disease, and are analogous to the periodic crops of generalized yaws. The local exacerbations have invariably been found to contain spirochætes; they must, therefore, be considered as true, active framboesic processes.

LOCAL OR REGIONAL LYMPHOGENIC METASTATIC YAWS IN PHILIPPINE MONKEYS

Contrary to the local exacerbations these lesions are detached from the area of the skin affected by the spreading initial yaw, from which they are separated by a more or less wide stretch of healthy skin which up to that time has not been involved in the yaws process. They occur, however, in the same part of the animal's body in which the local lesion established itself. They are of different aspect from the polypapillomatous manifestations of typical generalized yaws, being flat, very superficial, dry, spreading, and covered with a crumbling dry scab. They were observed once in our experiments, interestingly and not without significance in a case that exhibited unusually intensive local recidive (Plate 5, fig. 2).

The regional metastatic lesions are an expression, further advanced than the local exacerbations, of the inherent tendency of the framboesic process to become generalized, and to

achieve this generalization in successive attacks. Their distribution over the skin and their clinical behavior strongly indicate a lymphogenic rather than a hæmatogenic propagation. They are the forerunners of generalized yaws.

Generalized yaws manifestations are claimed to have been produced by Halberstädter(20) in an orang-utan and by Castellani(4) in a lower monkey. From the photograph of the lesion on the abdomen of an orang-utan it is difficult to judge. The photograph shows the entire animal, which makes the lesions proper too small to allow judgment. They may have been of the type under discussion. It is possible, however, that the lesion described by Castellani should be placed in this group of yaws lesions instead of being interpreted as the polypapillomatous stage of generalized frambœsia.

EXPERIMENTAL SUPERINFECTION AND REINFECTION WITH YAWS IN PHILIPPINE MONKEYS

Very little work has been done on superinfection and reinoculation of yaws in monkeys. Whatever experiments are recorded in the literature are mostly reinoculations, made for the purpose of testing the reciprocal immunity between yaws and syphilis. Neisser, Halberstädter, et al.(20) interpreted the failure of reinoculation in frambœsic monkeys as being a sign of immunity to yaws. Levaditi and Nattan-Larrier(13) interpreted their failure to infect syphilitic monkeys with yaws as proof of cross immunity. Ashburn and Craig(1) met with similar failure to reinoculate one Philippine monkey. It is an amazing fact that, notwithstanding there have been clinical observations of spontaneous autoinoculations as well as successful experimental reinoculations in human beings, the contrary doctrine that monkeys are not reinoculable with frambœsia has permeated the literature of yaws for the past twenty years. Instead of more-extensive experimentation having been undertaken, all possible theories have been invented to reconcile the discrepancies between the clinical observations and experiments on man and the findings in animals. This is not to be wondered at because, strange to say, the results of reinoculations of yaws in animals were uniformly negative and, as the experiments were performed at a time when much stress was placed on antibacterial immunity, the antitreponematous immunity was expected to be at once complete and protective. The failures in experimental reinoculation of yaws of twenty years ago and the occurrence of supposedly generalized yaws in anthropoid apes, which was disputed

by Ashburn and Craig,(1) who observed identical lesions in lower monkeys, are used as differential points in the discussions of the dualistic theory of syphilis and frambœsia. As recently as 1912 Plehn(25) stated at a meeting of the German Tropical Society that, with regard to animal experiments, "clinical signs of generalization were always absent, either in syphilis or in frambœsia, in lower monkeys."

Baermann and Schüffner(2) at the same meeting brought out as a differential point between the two diseases, the statement that "in higher species of apes secondary manifestations of yaws occur, as in syphilis; in lower monkeys, contrary to syphilis, these manifestations have not yet been observed."

The successful inoculation with yaws of Philippine monkeys, by Ashburn and Craig,(1) and their failure to transmit syphilis to these animals is given by Plehn(25) as another differential point. He says: "A particular species of monkeys, *Cynomolgus philippinensis,* is susceptible to frambœsia, but not to syphilis."

The periodic exacerbations of experimental yaws in animals which take the form of so-called "local recidives" and local metastases, the periodic crops of general yaws in human subjects, the gradual change of experimental primary yaws in animals from a papule (first stage), into a typical yaw (second stage), then into the ringworm form (third stage) and, finally, into the frambœsidelike, narrow, superficial lesion, coupled with some recent observations on human subjects, lead me to the belief that by superinfection and reinfection of frambœsic animals some of the manifestations of yaws observed in man may be duplicated in at least some of the experimental animals. Thus, it was hoped, the pathogeny as well as the immunity could be studied in animals. Experimentation on animals with respect to yaws has the advantage over observations and experiments on human beings that the problem can be divided into phases, which can be separately studied. In monkeys inoculated for the first time, as a rule, a primary yaw only develops. It runs its course, regresses, and heals without the yaws manifestation becoming generalized. This is of great advantage if one wishes to study immunity in yaws, as immunity may or may not develop as a consequence of initial local yaw. Furthermore, the pathogeny of certain generalized lesions can be studied with the view to discover whether they originate from a typical metastatic yaw or independently. In the attempt to approach the problem of generalized yaws and of immunity in yaws experimentally, observations were first made on numerous monkeys inoculated

for the first time. These observations were continued for a considerable length of time in order that information might be secured which would enable us to formulate one or more criteria for judging the effect of existing or healed yaws on the form of yaws lesions that develop in these animals upon superinfection or reinoculation.

By superinfection as here used is meant the artificial inoculation of viable treponemas into an animal that already has developed experimental yaws. The second inoculation is made at the stage when the yaws lesions, as a consequence of the first inoculation, are still present and either can still be shown to contain treponemas or can be reasonably expected to contain them even though they cannot be found by the methods employed.

Reinfection as used in this paper means the inoculation of viable treponemas into an animal that has gone through certain stages of a yaws infection, and in which the yaws lesions have disappeared as a result of specific treatment.

Furthermore, another object of these experiments was to study the immunity, if such exists, during the course of the artificial infection in monkeys or at the time of spontaneous or therapeutic recovery. Not only was the general immunity tested by infecting animals that had active or healed yaws in another place than that used for the first inoculation, but local immunity was tested as well by a second inoculation into healed parts of the skin of the animal over which the original yaws lesion had traversed. The viability of the treponemas in the inoculum was tested simultaneously on normal control monkeys.

I wish to mention again, and to emphasize the point, that up to the time when I began the superinfection experiments there was not a single case among the monkeys inoculated with yaws that would develop yaws lesions which might even be compared with the generalized yaws as observed in man. In that respect the findings of Ashburn and Craig(1) on Philippine monkeys have been fully confirmed. The typical yaws lesion as described above developed invariably at the point of inoculation only and, although the lesion gradually involved the surrounding tissue, it always spread by continuity, no metastatic lesion ever having been noted in other parts of the body. It was only when an extensive yaws lesion began to heal in such a manner that healed areas of skin intersected the original extensive lesion that an appearance was produced which easily might lead a casual observer, unacquainted with the history of

the lesion, to interpret it as a multiple lesion. Local exacerbations in such a lesion may strengthen this false impression, as has already been pointed out.

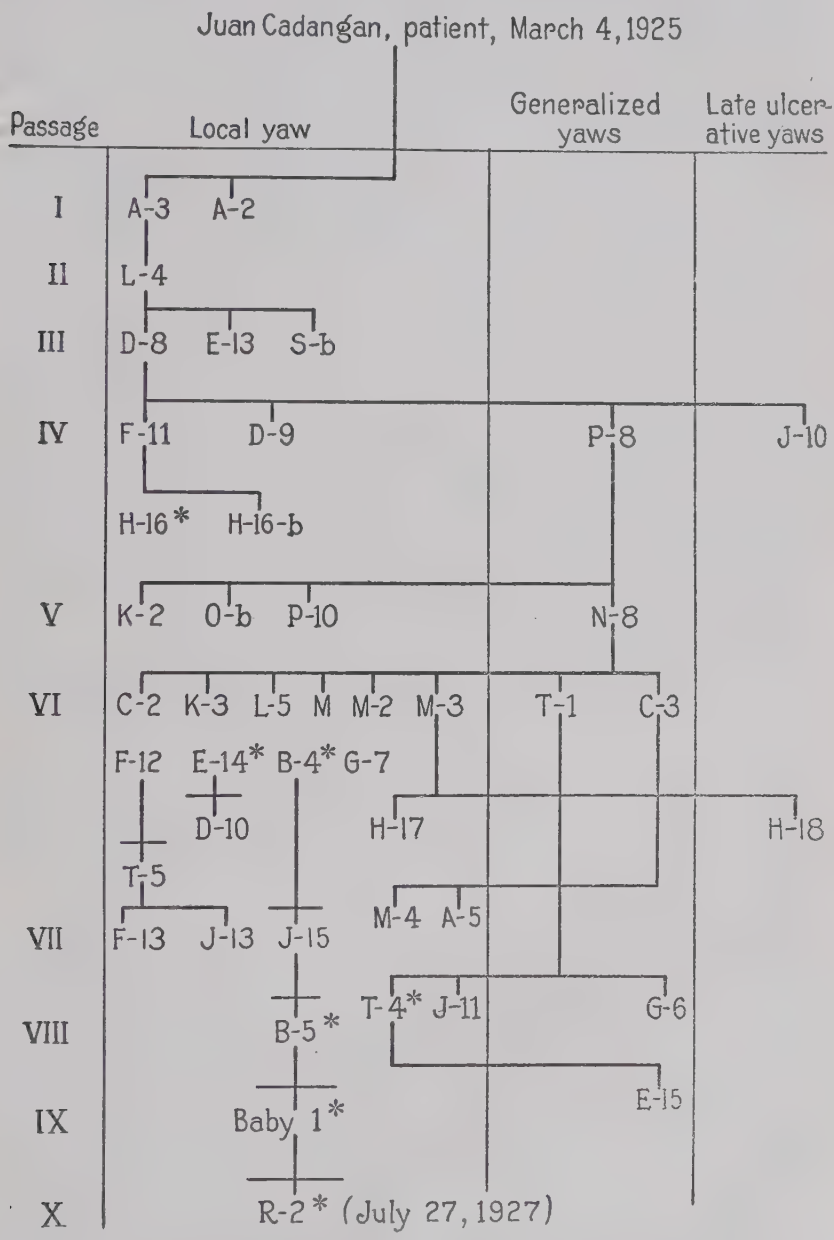

FIG. 1. Showing the results of superinfection and the continuous passage through monkeys of the Juan Cadangan strain of yaws. An asterisk indicates passage through monkeys without superinfection.

The animals were frequently seen scratching the lesions and pulling off the scabs, but in no instance did lesions develop outside of the original point of inoculation. In other words, spontaneous autoinoculation never was observed in these experiments.

It was not until superinfection experiments were started that typical metastatic multiple yaws lesions and late manifestations of yaws appeared in the superinfected animals, clinically and histologically identical with those found in man.

Text fig. 1 summarizes the continuous ten passages of the strain Cadangan through Philippine monkeys. It shows the number of local, generalized, and late ulcerative yaws produced in monkeys with this strain.

GENERALIZED HÆMATOGENIC YAWS, PRODUCED EXPERIMENTALLY IN PHILIPPINE MONKEYS

From the description of the primary yaw in monkeys it is evident, and should be emphasized, that only the superficial parts of the skin are affected; that, simultaneously with the spreading on the periphery of the lesion, the center heals as a rule completely, leaving the skin to all appearances normal. By repeated superinfection yaws lesions were successfully produced by me in some of the animals that were different in clinical appearance and course from those described as initial yaws. It was, however, nothing unusual to observe a lesion upon second inoculation which in no way differed from the typical initial yaw. The unusual lesions resulting from superinfection were of several kinds. First of all there resulted a diffuse, simultaneous, extensive, and very intensive local lesion over the entire nose and eyebrows or scrotum, with numerous treponemas. This local lesion was identical with the extensive local exacerbation and was followed shortly by a generalized eruption of typical yaws.

Typical metastatic yaws.—The typical metastatic yaw in monkeys commences as a small papule which spreads on all sides and soon becomes covered with more or less pronounced scab, due to the lymph oozing out of the lesion. The lesion is elevated, flat, sharply outlined and, as a rule, oval. It never reaches the size of the primary yaw, but in these experiments it was sometimes observed to assume a considerable size; that is, 0.5 centimeter by 1 centimeter (see Plate 6, fig. 2). When this size is reached the lesion becomes stationary and a central flattening is noticed which extends toward the periphery of the lesion. Thus the lesion heals gradually in toto, leaving a pigmented mark

that can be noticed long after the lesion has healed. Not infrequently, however, the metastatic yaw reaches not more than 0.5 centimeter in diameter and presents a dark crust set upon an elevated basis, which abruptly vanishes into the normal skin. Upon removal of this crust granulating, oozing, papillomatous formation is visible (Plate 6, fig. 1).

When the last-mentioned form of metastatic yaw becomes stationary, when it has reached the size of not more than 0.5 centimeter in diameter, it resembles in every respect the papular form of yaws as known in man.

Not always, however, does the metastatic yaw heal in toto, but frequently, after reaching the full size, it becomes stationary and sends off on the periphery a circular line of dry scabs that progresses on the periphery while the yaw proper is stationary. This manner of propagation brings about a concentric circular effect, as seen in Plate 16, fig. 1.

Ringworm yaws.—The ringworm form of metastatic yaws, frequently encountered in patients, could be studied from the early beginning to its full development and healing. The horseshoe effect or the semicircular arrangement of the ringworm yaw is due to the semicircular arrangement of multiple initial papules which, in growing, become confluent. This form of yaws experimentally produced in Philippine monkeys is shown in Plate 7. The lesion heals as a rule in toto, becoming gradually flattened and dry, leaving a slight pigmentation or none.

EARLY EVANESCENT FRAMBŒSIDES AND PSORIASIS PALMARIS IN PHILIPPINE MONKEYS

At least three forms of this early manifestation of yaws (evanescent frambœsides) have been noted in the course of these experiments.

The first form, a narrow circle of slightly inflaminatory lesions, consisting of a slightly elevated basis and covered with dry scabs, is shown in Plate 8. It begins as a typical papule and spreads out in a circular effect while it is healing in the center. When it has reached a certain dimension it becomes stationary, dries up, and disappears rather rapidly. In as much as treponemas have been found in these efflorescences, it can be considered as a mitigated form of ringworm yaw; but, due to its dermatological resemblance to the frambœside to be described next, it is here classed in the group of frambœsides.

The second form is a diffuse desquamative superficial frambœside. It occurred first on the front part of the neck, on the

inner surfaces of the extremities, and then migrated gradually over the entire chest and abdomen. It is illustrated on Plate 13. The individual efflorescences had a typical maplike, circinated shape. There was no reaction at the basis, or only a very slight one. By confluence of the maplike individual efflorescences there resulted an effect not unlike that of dry seborrhœa. No treponemas could be found in this skin lesion, and its affiliation with yaws is based on the ringworm shape of the lesion and on the prompt healing, on neosalvarsan therapy, and by elimination. No such skin affection was noticed on examination of more than two hundred normal Philippine monkeys, either newly secured or kept in captivity for a long time.

The third form of early evanescent framboesides observed in Philippine monkeys was psoriasis palmaris and psoriasis plantaris. The first of these is shown in Plate 10 and is not quite typical as it is on the border line of the framboeside mentioned as first in these enumerations and contained treponemas. The unusual feature is the grouping of the lesions into a circinated design. The tendency to symmetric distribution was marked but fell short, in as much as the corresponding lesion on the opposite arm appeared on the wrist instead of on the palm. Because of its peculiar grouping, it is not an exact duplication of psoriasis palmaris as this condition is commonly found in man, but resembles it more than any other palmar lesion known to me, particularly when the individual efflorescences are considered rather than the entire lesion. The psoriasis plantaris shown in Plate 12 is typical. No treponemas could be found in this lesion. The initial lesion was noticed to be a flat, elevated macule of dark color, which gradually spread in the periphery until it reached about 0.25 centimeter in diameter. The central portion of the epidermis, which up to that time had remained intact, began to dry and exfoliate. The exfoliation was very superficial and circular, outlined by a paper-white rim. The basis of the exfoliated center was dry, did not ooze, and showed no scab formation. The exfoliation proceeded faster than the spreading of the elevated macule and, after reaching a certain size, the lesion regressed and disappeared while new lesions made their appearance. The successive lesions migrated over the entire sole of the foot, including the pads of the toes. A definite symmetric distribution on the soles of the feet was noticeable.

The affiliation of this type of lesion with yaws is based, first,

on its great resemblance to psoriasis palmaris framboesica in man and, second, on the prompt healing due to specific treatment.

The typical course of human yaws one is accustomed to read about in textbooks can be best seen in Philippine monkeys from the record of monkey P–8. Following superinfection this monkey developed typical, generalized, metastatic yaws on the wrist, elbows, in the gluteal region, and on the legs. The lesions went through the usual course of the metastatic yaw and in time disappeared, leaving a dark pigmentation. Shortly after the general eruption occurred and while this was still present, there appeared lesions in various parts of the body in crops of rather protean character. During the entire course of the general infection a diffuse alopecia developed in the parietal regions, which gradually became more extensive and involved other parts of the body, particularly the tail. The generalized lesions encountered on the elbows were not unlike psoriasis, except that the silvery scales characteristic of psoriasis were absent and slight oozing rather than bleeding occurred on removal of the scales. When the general metastatic typical yaws started to regress and while still present there appeared circular, ringwormlike, dry efflorescenses with a distinctly inflammatory basis in the plicæ cubiti. These symmetric lesions disappeared without a trace and a crop of isolated, circinated, superficial efflorescences with branny desquamation followed. These efflorescences gradually became confluent, simulating dry seborrhœa. While this eruption was still present there appeared on the tail a crop of flat, elevated, papular efflorescences, partly desquamating on the periphery (ichthyosis). The general condition of the animal with generalized yaws became worse, until extreme marasmus set in. Keratoderma plantare of slighter degree than as illustrated in Plate 24, fig. 2, developed toward the end but did not reach the exfoliative stage. Specific therapy brought about prompt disappearance of the lesions, but the animal died. The general characteristic of the successive crops of yaws eruptions in monkeys is that the succeeding crops are more superficial than the preceding ones.

Aside from the typical mother yaw, and the typical metastatic and the ringworm yaws, the skin lesions just described must be considered as framboesides. In some of them the presence of treponemas was demonstrated by the dark field, in others they could not be found. This brief summary of the generalized yaws

in Philippine monkeys presents the entire picture of so-called "primary," "secondary," and late yaws well known in man. The ulcerative lesions met with in late yaws in man did not develop on one and the same monkey, but they developed in other monkeys that had not gone through a generalized eruption of typical metastatic yaws. Generalized yaws lesions were claimed to have been produced before on the abdominal skin of an orang-utan by Neisser et al.(13) Ashburn and Craig(1) noticed similar extensive yaws lesions in a Philippine monkey. Having compared the photographs of Neisser et al.(13) with the lesions we produced and which we consider as generalized, we agree with Ashburn and Craig(1) who say:

> The new lesions were easily demonstrated to be extensions, in direct continuity with preëxisting ones and sound skin was never found separating these lesions while in the active stage. Their progress answered perfectly to the so-called secondary lesions described by Neisser, Baermann, and Halberstädter, but as we have stated, we can not regard them as an evidence of a general infection and therefore as "secondary" in the sense in which the term is used in connection with syphilis.

Ashburn and Craig(1) further state emphatically:

> * * * we believe we are justified in asserting that in the species of monkey used [*Cynomolgus philippinensis*] a general eruption of yaws does not occur after experimental inoculation.

These authors evidently do not believe that either their case in a Philippine monkey or that of Neisser et al.(13) in an orang-utan was true general yaws. They, furthermore, express their opinion very reservedly with regard to the experiment of Castellani(3) for they say:

> Castellani appears to have secured true secondary lesions situated at a distance from the original papule in his one successful inoculation and in this case *a general infection might be supposed to exist.*[4]

These lesions appeared in the face at a short distance from the original initial local yaw. A photograph not being available I am not in a position to pass definite judgment on the lesions produced by Castellani;(3) but, from the description quoted by Ashburn and Craig,(1) I believe that they were local lymphogenic metastatic lesions rather than the so-called "local recidives" and, therefore, similar to my case (Plate 5, fig. 2).

The question whether or not yaws in Philippine monkeys can be induced to run the same clinical course as it does in man finds its answer in this section. All previous experimenters

[4] Italics mine.—O. S.

agree that the primary efflorescence, or the mother yaw, is so typical that it can be recognized in monkeys as such. Ashburn and Craig(1) state:

> All * * * monkeys developed yaws lesions of sufficiently characteristic appearance to permit of diagnosis based on that feature alone. [p. 451.] The lesions [primary] in monkeys do not differ essentially in their morphology from those occurring in the disease in man, * * *. [p. 457.] We believe from their [primary lesions] appearance alone a clinical diagnosis could be made even in the mildest case of infection we have observed, while in the more severe infections * * * the nature of the lesion was apparent at a glance. [p. 460.]

As to the course of the primary lesion in Philippine monkeys, Ashburn and Craig(1) state: "After persisting for varying periods of time the lesions of frambœsia heal in the same manner as those occurring in man." The similarity in development, course, and healing of the experimental initial yaw in monkeys to those in man is generally recognized. Unfortunately, previous experimenters did not succeed in producing any further lesions of yaws in animals. Anyone who has seen the development and course of generalized yaws in monkeys will be impressed by the fact that the typical metastatic yaw, in monkey as in man (Plate 9, fig. 1), is far more characteristic than the initial local yaw, and no one who has seen the multiple, typical, metastatic yaws in man will hesitate to recognize them in animals. There is no doubt that it was the appearance of the metastatic yaw, not that of the mother yaw, that suggested the name frambœsia (raspberry).

The lesions, just discussed, in the superinfected monkeys and in the single-inoculated monkey (see below) are considered metastatic and not accidental autoinoculations, for the following reasons:

1. They were never noticed in animals infected for the first time and in which the infection ran the usual course of the local experimental yaw.

2. They were found exclusively in animals that developed an unusual extensive and intensive local lesion at the place of superinfection, or that showed an unusually extensive and intensive exacerbation (Plate 3 and Plate 5, fig. 1).

3. They did not resemble the initial local yaw but did resemble metastatic generalized yaws in man, and they occurred in successive crops.

4. They were symmetric, and were located topographically in parts of the animal's body where all attempts at artificial

inoculation persistently failed and, strangely enough, seldom if ever occurred in places where local yaws lesions could be produced with ease.

TOPOGRAPHY OF GENERALIZED YAWS

Definite regularity was noticed in the distribution of the metastatic lesions in animals used in these experiments. The early generalized lesions (that is, the typical metastatic yaws and the metastatic ringworm forms), both skin lesions in which treponemas were readily demonstrable, were, briefly speaking, juxta articular. The dorsal surface of the wrists, elbows, plicæ cubiti, ankles, knees, and trochanters were the places of predilection. The lesions were symmetric though not always located on identically corresponding places.

The early evanescent circinated frambœsides migrated in successive crops over the front part of the neck, the chest, the abdomen, and the inner surfaces of the extremities.

SPONTANEOUS GENERALIZATION OF YAWS IN A PHILIPPINE MONKEY FOLLOWING A SINGLE INOCULATION WITH TREPONEMA PERTENUE

In the later part of this experimental work[5] I had the opportunity to observe a spontaneous generalization of yaws infection in a monkey following a single inoculation. This singular case seems to warrant more-detailed description. The sequence of the lesions and the course and development of the spontaneous general infection proved the correctness of the plan conceived with the view to produce general clinical manifestations of yaws in animals by repeated superinfection. It also explains the success attending these experiments, in as much as the course of the natural general infection was a duplication of that which I observed in other animals as a consequence of superinfection. The objection therefore that, under such artificial experimental conditions, the general infection in yaws monkeys may assume a course unnatural with these animals lacks all grounds.

A rather strong male Philippine monkey was inoculated on December 11, 1925, with material containing numerous treponemas. The material was obtained from a metastatic typical yaw in a monkey that had been superinfected. After the incubation period a distinct oozing lesion on the right inner eyebrow and

[5] The experiments were commenced on August 12, 1924.

a slight swelling on the left eyebrow, as well as a distinct flat papule measuring 0.5 centimeter by 0.25 centimeter, covered with moist scab, developed on the scrotum by January 4, 1926. These three points (that is, both eyebrows and the scrotum) were the places where inoculation was performed. The yaws ran the usual course and nothing out of the ordinary was noticed until February 3, 1926, when an extensive local exacerbation was noticed on the eyebrows, the nose, and the scrotum (see Plate 3). The spreading original lesion on the eyebrows had reached the nostril, as is evident from Plate 4. On March 31, 1926, the lesion shown in Plate 5, fig. 2, was first observed. It was a yaw separated from the original mother yaw by healthy skin, but located in the same part of the animal's body as the mother yaw with the excessive local exacerbation. In a previous section it was mentioned that this lesion was interpreted as a lymphogenic or local metastasis.

On account of the extensive local exacerbation which developed on the eyebrows and the scrotum, and due to the appearance of the local metastases, the monkey was purposely not superinfected and the infection was allowed to run its natural course, in the hope that a spontaneous generalized manifestation of yaws might take place. This hope was realized when, on April 27, 1926, metastatic lesions were observed on the elbows, the dorsal part of the wrist of the right hand, and the palm of the left hand (Plate 10). It will be noticed that the original mother yaw, as a result of the artificial inoculation of the monkey, lasted from January 4 to April 27, 1926; that is, approximately three months and three weeks elapsed before the real general manifestations appeared. The local lesion noticed at the time of the general eruption, however, was the exacerbation and not the original lesion which had regressed and spread by that time as a thin line of dry scabs beyond the area covered by the recidive. This fact confirms the correctness of the idea that led me to adopt the plan of repeated superinfection in monkeys; that is, if the local yaw were to last long enough or could be artificially maintained long enough, a certain percentage of the experimental animals should develop one or the other manifestation of generalized yaws. The extensive local exacerbation in this case took the place of the artificial superinfections by means of which in other animals generalized manifestations were produced.

In as much as the lesions on the eyebrows and nose had reached the margin of the nostrils and all the lesions contained

fairly numerous treponemas, no superinfection was made after the appearance of generalized yaws, so that it might be learned whether or not the lesion on the nose would spontaneously last until the allergic state had been reached, which is responsible for the formation of deep ulcerative lesions; in other words, whether or not spontaneous gangosa would develop in this animal. However, my previous experience with superinfection in monkeys that had gone through generalized yaws led me to expect that the immunity would rise rather rapidly and that the manifestations of general yaws would disappear without having become ulcerative lesions.

During the regular inspection of inoculated monkeys a new crop of lesions in the animal under discussion was noticed on the lower extremities on June 5. On the plantæ the lesions were macular, consisting of irregularly outlined macules, which were slightly elevated, darker in color, and about 0.5 centimeter in diameter. They were located mostly on the soft skin of the instep, on the sole of the foot, and on the terminal pads of the toes. They were symmetric to the extent that the corresponding toes on both feet were similarly affected, as were also the soles of both feet. There was noticeable a very superficial exfoliation. The exfoliated part showed no crust forming and no oozing. There was no thickening of the skin, and the lesions started to regress within a few days and new ones appeared (see Plate 12). Along the lower part of the calf in the vicinity of the ankle lesions were noticed of circinated papular efflorescences with a red basis and topped by silvery scales, not unlike those in lichen planus. The lesion on the left hind extremity was circular and measured 1 centimeter in length, and lower down were two papules of the same character. The main lesion was horseshoelike in shape, and within the space half surrounded by the lesion were minute papules with a red base and silvery scales (Plate 8). On the right lower extremity in a corresponding place four small papules were noticed at this time, distinct but grouped in a semicircle. They were of the same character as the lesion on the left hind extremity already described. A circinated scaling occurred symmetrically on both forearms on the extensor side, consisting of distinct, small papules, covered with silvery scales. On the left wrist, on the radius side right over the joint of the thumb, was a typical yaw covered with dry crust, measuring 1 centimeter in diameter. The surrounding basis was elevated, reddish, and the crust was solid and dry, rather tenacious, but crumbling when forcibly removed,

and leaving a slightly bleeding and oozing base. From that time on the lesions, both metastatic and local, began to regress. On June 17 the lesions on the face and on the scrotum still contained very numerous active treponemas. The lesions on the soles of the feet had regressed considerably by that time, but some superficial desquamation was still present. The yaws over the wrist had spread out in a circinated effect, and the one on the leg had practically disappeared. By July 12 the face had practically healed. There was a remnant of dry crumbling scabs on the tip of the nose and in the nostrils.

On July 24 a remnant of dry scales was visible on the nose. The entire area previously affected by exacerbated yaw showed pigmented and depigmented spots. The nostrils were perfectly clear. On the scrotum there were still a few small lesions, some of them covered with scab. The entire scrotum was darkly pigmented. There was a very slight desquamation on the left wrist and on the left palm, also on the plantæ pedis. The rest of the metastatic lesions had disappeared, leaving no trace.

On July 30 a few remnants of lesion on the scrotum were noticed. The lesion on the left wrist still persisted as did also the slight desquamation on the plantæ. The face was healed, not only the original lesion, but also the lesion on the left jaw.

On August 6 the lesion on the scrotum was found to be in the same condition as before. On the hands the lesions had practically disappeared except on the palm of the left hand where some scaling was present. The nostrils were clear. Dark-field examination showed fairly numerous treponemas in the lesion on the scrotum.

On August 11 small scabs still persisted on the nose, and there were slight alopecia over the eyebrows and a few scabs drying up on the scrotum. Of the generalized eruption very slight scaling remained on the left palm and on the left wrist, accompanied by alopecia on the elbows. The areas of healed generalized eruption were evident by pigmentation. There was some scaling on the soles of the feet. Treponemas were found in the lesion on the scrotum.

On August 18 a very few small lesions were found on the pigmented scrotum. The face had completely healed. Fairly numerous treponemas were found in the lesion on the scrotum.

On August 30 there was nothing but pigmentation on the face. There were also pigmentation and scars on the scrotum. The arms, the hands, and the tail showed no signs of yaws.

INCUBATION PERIOD AND DURATION OF GENERALIZED FRAMBŒSIC PROCESS IN PHILIPPINE MONKEYS

It has been mentioned that the average incubation period of a local yaw from the time of inoculation to the appearance of the lesion recognizable clinically as yaw was, on the average, twenty-six days. Compared with the incubation period of local yaw in artificially inoculated volunteers,(30) it can be seen that this agrees with the incubation period in man, which was found to be three and one-half to four weeks upon first inoculation and three and one-half to six weeks upon second inoculation of the same volunteers. If the incubation period of the generalized yaws in our experiments performed on humans is compared with that in experiments on Philippine monkeys it will be found that the interval between the first inoculation and the appearance of generalized yaws in man was between ten and fifteen and one-half weeks. In the monkey that developed spontaneous generalization after a single inoculation the incubation period of the generalized yaws was three months, or twelve weeks. It can be seen, therefore, that the incubation period of both the local and the generalized yaws agree very well with the period found experimentally to exist in humans. In the rest of the monkeys that showed generalization upon superinfection the findings were as follows: The incubation period of the generalized yaws from the first appearance of the local yaw due to the first inoculation up to the appearance of generalized yaws was on the average eleven weeks, four weeks having been the shortest and twelve weeks the longest incubation period found. However, if the incubation period is calculated from the time that extensive local lesions occurred as a result of superinfection, it will be found that this incubation period was on the average three and eighty-five hundredths weeks, two weeks having been the shortest and seven weeks the longest period. Up to this stage the course of generalized yaws agrees very well with the incubation period observed in human volunteers. The duration, however, of the generalized process (that is, the period of time from the first occurrence of the generalized manifestation until complete disappearance of the last lesions) differs from that in humans. The average duration in monkeys is only three months, or twelve weeks. In other words, the duration of the generalized yaws in monkeys is apparently much shorter than the average duration of the generalized yaws in man. It averages in monkeys about as many months as it does years in humans.

MORPHOLOGIC AND CHRONOLOGIC RESEMBLANCE BETWEEN THE DEVELOPMENT, COURSE, AND HEALING OF THE MOTHER YAW AND THE METASTATIC PROCESS OF GENERALIZED YAWS, INCLUDING THE FRAMBŒSIDES, AS OBSERVED IN THE COURSE OF EXPERIMENTAL YAWS IN PHILIPPINE MONKEYS

With the picture of the stages in development, course, and healing of the initial local yaw fixed in mind the interpretation of the polymorphous generalized lesions is not so difficult as one would believe at first when confronted with these lesions, some of them changing so constantly that they well deserve the epithet "protean." The frambœsides and the typical polypapillomatous manifestation, as is the case with the initial local yaw, commence with a more or less pronounced papule (first stage of the mother yaw). The typical metastatic generalized yaw develops, further, into a vigorous healthy yaw (second stage of the mother yaw), without going into the third stage of the course of the initial local yaw; it usually stops there, flattens, and heals in toto, leaving a pigmented trace within the entire area affected. Sometimes, however, it becomes stationary and sends off a narrow line of dry frambœsidelike lesions.

The ringworm yaw follows the same stage of development as a typical local yaw, but it starts as multiple papules arranged in a semicircle. This arrangement of the initial papules is responsible for the horseshoe shape of ringworm yaw, which arrangement is maintained more or less throughout the entire course of the lesion.

The ringwormlike frambœside commences as a papule and develops into a small, flat, rather dry, mitigated yaw which spreads on the periphery presenting, first, a seamlike, circinated design. However, the central healing progresses as fast as does the peripheral spreading and the result is as shown in Plate 8. After it has reached this stage the lesion disappears.

The ringwormlike frambœsides follow, therefore, the first stage (papule), a mitigated second stage of the local yaw, and the ringwormlike third stage of the initial local yaw.

The early evanescent, desquamative, migratory frambœsides (Plate 13) start as hardly perceptible papules (first stage of the initial local yaw) and, omitting the typical yaw and the ringwormlike stage of the local yaw, change immediately into the vanishing lesion of the mother yaw. They appear as the last number in the repertory of general early frambœsides, and because of the pronounced exfoliation they constitute morphologically a transitional stage to the ichthyotic, exfoliative, per-

sistent late frambœsides (compare the fifth and the sixth stage of the mother yaw) of which the keratoderma plantare frambœsicum is a local exaggeration.

The individual stages in the development, course, and healing of the local yaw, as manifested by the morphologic changes of the clinical lesions, are reflected in the various forms of generalized yaws manifestation. It is true that certain generalized yaws lesions designated as late manifestations may occur much earlier in one case than in another, but in a single given case the tendency of the manifold frambœsic lesion is to occur in a certain sequence which follows in a general way the sequence of the changes in the course of the local yaw; that is, the general manifestation begins with the eruption of typical or ringworm yaws. Then follow the ringworm frambœside, the maculo-papulous plantar or palmar frambœside, the superficial desquamative frambœside, the ichthyotic desquamative lesion, and the hyperkeratotic manifestation. As in the local yaw, in the metastatic manifestation the early eruptions are of short duration and either occur in crops or migrate over the skin, while the desquamative and hypertrophic manifestations are more persistent.

LATE YAWS MANIFESTATIONS IN PHILIPPINE MONKEYS

ULCERATIVE FORM OF THE SKIN LESIONS

The ulcerative form of lesions was observed in a limited number of superinfected animals. They had had local yaws lesions for a considerable length of time previous to the superinfection. An illustration of this type of lesion, as it developed at the place of superinfection on the left ala nasi, is given in Plate 17, fig. 1, and on the eyebrow in Plate 22, fig. 1. These lesions represent sharply outlined, rather deep defects. They spread slowly, affecting the entire thickness of the skin. In contrast to early lesions, they showed no tendency to central healing, but ultimately healed from the periphery by scar formation. The oozing so characteristic of early lesions is not so well pronounced and the crust is dark, flat, and dry.

GANGOSA

Gangosa, so-called, is a condition that appears to be peculiar to the inhabitants of tropical countries; its relation to yaws was early recognized by a number of observers.(24) That view as to the etiology of the condition has not, however, met with unani-

mous support in the more-recent literature. It has been asserted that, in cases of gangosa, syphilitic and other systemic infections have not been excluded. Others who have studied the problem have reached the conclusion that gangosa is a disease sui generis.

Manson-Bahr(16) gives the following definition of gangosa:

> Gangosa or destructive ulcerous rhinopharyngitis, which has been regarded by some as a sequel of yaws, generally commences as an ulcer on the soft palate. Slowly spreading it may make a clean sweep of the hard palate, the soft parts, cartilages and bones of the nose, sparing the upper lip, which is left as a bridge across a great chasm, the floor of which is formed by the intact tongue.

As to the etiology Manson-Bahr(16) states:

> The lesion has been attributed to leprosy, tuberculosis, syphilis, and yaws. There is *no satisfactory evidence* that it is due to any of these diseases.

Farther on he states:

> The cases the Editor has personally observed were associated with tertiary yaws-scars on the other parts of the body. It has been suggested by Leys that gangosa is a disease sui generis; there is no conclusive evidence for this at present. On the other hand, it is not a form of buccal leishmaniosis.

Musgrave and Marshall(19) express their opinion with regard to gangosa thus:

> * * * we will merely state that we have never seen any lesions of yaws which presented the faintest resemblance to gangosa, although the typical framboesial skin lesions are not uncommon in this region [Philippines]. [p. 398.]

These authors state:

> Mink and McLean, * * * Leys * * * satisfied themselves that there is no causal connection between syphilis and gangosa. Mink and McLean also convinced themselves that the disease is independent of yaws. Fordyce and Arnold excluded syphilis from the diagnosis of their case. [p. 399.]

Musgrave and Marshall(19) also think that—

> * * * the weight of evidence is in favor of the view that gangosa is a disease independent of syphilis, but we do not regard this as a definitely established fact. [p. 399.]
>
> It will require careful observations on several cases * * * to justify a positive expression of opinion as to the part played by syphilis or yaws in the gangotic process. [p. 400.]

Castellani and Chalmers(5) state: "It is quite possible that gangosa is really a tertiary manifestation of yaws affecting the palate and nose."

Plehn and Mense(6) see only two etiologic possibilities of gangosa—syphilis or yaws. The latter seems to them more likely than the former. As late as 1926, according to H. S. Stannus,(31) "the etiology of this condition remains unsettled."

I have had the good fortune to produce and observe the development of gangosa in Philippine monkeys, animals known to be free in their natural state from yaws or any other similar infection, either hereditary or acquired.

The pathogenesis of one type of gangosa as it developed in the experimental monkeys was as follows: The typical initial local yaw of the eyebrows spread down the nose and cheeks, finally reached the nasal mucous membrane, and spread by continuity directly thereon. At this stage the skin lesion healed and completely disappeared, while the lesion on the nasal mucous membrane persisted. However, the fact that a spreading local yaw lesion has established itself on the mucocutaneous border of the nose is not alone sufficient to produce the mutilating destructive process of the soft parts of the nose as is known to take place in gangosa. The allergic state is necessary for an accomplishment of this destruction. I have repeatedly seen an initial local yaw, in monkeys infected for the first time, descend from the eyebrows over the nose in a butterfly effect and enter the nostrils, where it lingered for some time after the lesion in the skin of the face had healed. Ultimately, however, it healed also without producing any destruction of the nasal structures, leaving, at the worst, only a superficial skin scar that extended to the mucocutaneous border. The slight cicatrization resulting from a termination of the local yaw in a condition of the body organism unfavorable to the development of a mutilating process reminds one of the scars on the upper and lower lips found in healed yaws patients. Such scars, indeed, are not infrequently found in cases of gangosa in man, and indicate that yaws have entered the mucous membrane of the nose directly from the lips of the mouth or from the skin around the nostrils.

In the case of experimental gangosa under discussion, superinfection was performed on the eyebrow. In due time a rather deep, sharply outlined, ulcerating lesion developed on the eyebrows. Simultaneously, the residual lesion on the nasal mucous membrane exacerbated and a mutilating ulcerative lesion

developed on the inside of the nasal cavity, affecting the septum and spreading backward without at first affecting the skin of the nose. As a matter of fact, the perforation of the septum was accomplished long before the ulcerative process had affected the alæ at all (Plate 22, fig. 1). Subsequently the alæ also were included, but up to the end no involvement by the later ulcerative process of the skin surrounding the nose was noticed (Plate 23, fig. 1). The anatomic conditions within the nose, as they existed at the time of the death of the animal, are evident from the photograph (Plate 25, fig. 2).

The other form of gangosa is illustrated in Plate 24, fig. 1. In this case a deep ulcerative lesion developed at the point of superinfection; that is, on the left ala nasi (Plate 17, fig. 1). The destruction progressed directly through the ala nasi and spread inwardly as well as onto the surrounding skin (see Plate 23, fig. 2). Unusually pronounced granulations, filling the nostrils, were found in this case; so exuberant were they that at times the breathing of the animal was rendered difficult. Both of these varieties of gangosa (that is, the nasal and the nasocutaneous) are found in man, and there is no doubt that their pathogenesis is the same as that given above.

A papule or an ulcer on the soft palate is designated as the primary lesion in gangosa, by Manson-Bahr(16) and by Plehn and Mense.(6) This seems to be the generally accepted view, for Castellani and Chalmers(5) say: "It appears to begin sometimes as a sore throat or coryza, or as a tubercle on the palate." This assumption necessarily presupposes a metastatic origin of gangosa, because it is hardly conceivable that an initial local yaw would originate on the soft palate. How this conclusion as to the original gangotic lesion, expressed in all handbooks and textbooks in monotonously uniform language, was arrived at was not clear to me at first. I failed to find in the literature any record of clinical observation of the course of development of gangosa, from the very early to the well-pronounced stage, or any experimental evidence concerning gangosa, either in man or in animals, that would support such a contention.

In his classic description of this disease J. Numa Rat(24) says that gangosa "often, however, avoids the nares and commences in the soft palate. The ulceration of the tubercle [granuloma] extends thence, destroying the uvule and *velum palati,* and the *septum nasi.*"

This does not necessarily mean that the lesion did not commence in the nose. As a matter of fact, our experimental case in monkey J–10 (Plate 22, fig. 1) showed perforation of the entire anterior septum at a time when nostrils and alæ nasi were still intact. In spite of the findings that the external nose and the surrounding skin of the face at the time when this photograph was taken were intact, previous records and photographs of the same animal (Plate 15, fig. 1) show that the lesion entered the nose by extension of the framboesic skin lesion onto the nasal mucous membrane. There is nothing in the summary of the observations on gangosa by Numa Rat(24) that is contrary to our conception of the pathogeny of gangosa as given above.

Another reference to the initial lesion of gangosa is that of Leys(14) the author of the technical term rhinopharyngitis mutilans.

Leys says:

CLINICAL SIGNS AND SYMPTOMS

The usual history of a case of this disease is as follows:—The patient, if seen early, as few are, complains of *sore throat.* On examination *an ulcer* is seen *on the back of the pharynx,* on *a posterior faucial pillar,* or on the free *edge of the palate.* It is *superficial,* moveable, *covered with a thin, dirty, brownish-grey pellicle of slough. This appears to be the initial lesion.* The pellicle breaks down and *leaves an ulcer which steadily increases, advancing up the throat into the posterior nares.*[6]

There is nothing particularly characteristic in this description of the initial lesion of gangosa. It may apply to diphtheria just as well as to syphilis. Yaws can be excluded (1) in "those seen regularly one case aged 2 and one aged 4 died, and another 9 carefully observed and treated for several months," (2) on account of the exclusive location of the lesion on the mucous membrane; (3) and the statement that "none of the three cases of children of 3, 4 and 9 showed any evidence of having had yaws."

It is difficult to say definitely from what affliction these youngest (therefore earliest) supposedly gangosa cases suffered. The sole survivor of these patients "improved steadily," but neither the nature of the treatment nor that of the improvement is given. In the absence of individual clinical records, bacteriologic and histologic examination, and autopsy findings, it is safe to say that syphilitic "plaques muceuse" can be eliminated on account of the youth of the patients.

[6] Italics mine.—O. S.

The paper of Mink and McLean,(18) appearing in the same year as that of Leys(14) and originating from the same institution and from the same small island, Guam, throws some light on the question.

Mink and McLean(18) give the following early symptoms of gangosa:

SYMPTOMS

Onset.—Only three cases have been observed from the earliest stages of the disease. The majority of patients present themselves for treatment only after mutilation is marked. In the three cases above mentioned the initial symptoms led to a diagnosis of tonsilitis, pharyngitis and laryngitis of mild degree. The patients were between the *ages of 12 and 15 years* and were in good physical condition. *Prostration* was slight or absent. All showed a slight *rise of temperature* and complained of *soreness in the posterior nares and pharynx,* with *stiffness of the muscles of deglutition. In one* case a typical *acute coryza* was present. Inspection at this time showed mild *congestion of tonsils, pillars, soft palate and uvula.* During the first week the general condition was practically unchanged.

Local symptoms:—The throat symptoms became localized, and on the *third day a patch of yellowish-gray membrane* was observed *on the soft palate in the first* case, *the uvula in the second* and *the right pillar in the third case.* The *membrane* was *elevated, thick* and *extremely tenacious.* On removal the denuded *surfaces bled freely. Within twenty-four hours* of the appearance of the membrane the *typical* ulceration was established. On the area covered by the membrane a number of small depressions appeared. The ridges between the depressions and the membrane were rapidly absorbed. *The ulcer,* now about one-half inch in diameter, *had a punched-out appearance,* with *undermined edges and a deep, uneven floor, covered with a yellowish-white very offensive discharge.* It was *surrounded by a zone of inflamation* about one-fourth inch in width. The *depth of the ulcer rapidly increased* until, in the case of the uvula and soft palate, *the tissues were perforated by the seventh day.* These cases are still under observation.[7]

If there remained the slightest doubt in the mind of the reader that the affliction described in the papers of Leys(14) and of Mink and McLean(18) as initial gangosa was anything but diphtheria (nasopharyngeal), a common form in the Philippines, it will be thoroughly dispelled by the following paragraph in the paper of Mink and McLean:(18)

Fulminating Gangosa:—This type *occurs in children under 5 years of age,* and all cases seen have been in gangotic families. In some instances *two or three children in the same family have been attacked.* The *sudden onset, extreme prostration, extensive membrane, marked cervical adenitis and rapidly developing toxemia and dyspnea* give a picture closely *resembling diptheria. Death occurs from toxemia* rather than from dyspnea. *The great majority of these cases prove fatal* within *forty-eight hours.*[7]

[7] Italics mine.—O. S.

It is a pity that, in the absence of laboratory facilities to eliminate diphtheria, antitoxin was not administered. This measure would have decided the etiology of the disease and would have saved the history of medicine the burden of a new term (fulminating gangosa).

There is no intention on our part to deny the fact that gangosa is prevalent in Guam; it always was. In point of fact, the Spanish government of the Philippines saw fit in 1828 to send a commission to the Carolines, of which islands Guam is one, to investigate the question. Two extremes have been pictured by Leys(14) and by Mink and McLean,(18) and a wide gap yawns between them. These authors have painted the vivid and sometimes tragic picture of the initial stage with its high mortality; then they transport us abruptly to their observations on well-developed cases of real gangosa—a disease by which "at no time is the patient's general health materially affected." The intervening events do not fall within our vision; we have passed over a gap that cannot be bridged by any reasonable stretch of the imagination.

It is realized that the foregoing may provoke the charge that the criticism of these two papers is unnecessarily extreme; that the simple citation of references would have sufficed; and that there was no need of the lengthy quotations. However, I have criticized and quoted at length for what seem to me good reasons and dare to hope for much in consequence. During a period of twenty years these papers have been quoted as standard references and, in parallelism, for a corresponding period the initial lesion of gangosa has been regarded as an ulcer or membrane on the palate. The textbooks and handbooks have told us this with an offset in the dogmatic statement that yaws processes never occur primarily on mucous membranes. It is my belief that these publications have assumed a heavy responsibility for the regrettable fact that gangosa, during all these years, has not been granted admission into the frambœsia family, where it rightfully belongs and where it was placed by Numa Rat(24) before 1891 and by other experienced frambœsiologists since.

It is earnestly hoped that a revision of the question of initial lesion and pathogeny of gangosa be made. More-recent observations on patients seem to give some indication of the correct pathogeny of this condition, and experimental findings on animals points only one way.

Rhinopharyngitis mutilans, the name bestowed by Leys(14) upon this form of yaws, is, as its author admits, a descriptive term, and the name gangosa (Ruis de Villalobos) is preferable. It occurs in the literature since 1828 and no doubt has the priority right. It is a local Spanish name that does not mean muffled voice as noticed in sore throat, diphtheria, or quinsy, but is like the Tagalog name ñgon-ñgon, imitative of the speech of a person with a perforated palate. By remembering the name gangosa, the student of tropical medicine will be reminded that what is meant by that name is not rhinopharyngitis mutilans due to syphilis, lupus, leprosy, cancer, leishmaniosis, or rhinoscleroma, but the condition caused by frambœsia tropica and nothing else. He will, of necessity, by knowing this name, remember the geographic distribution of the disease.

In the light of our experimental researches the only explanation for the claim that the starting point of gangosa is the soft palate would be this: When metastatic yaws establishes itself on the soft palate, it lingers there for some time, as the primary yaw did in our experiments on the mucous membrane of the nose. It persists there until the reactivity of the body becomes favorable for late ulcerative lesions. Initial or metastatic yaws on mucous membranes are not known to exist; but direct spreading of primary or metastatic yaws from the skin across the mucocutaneous border onto the mucous membrane is by no means an uncommon occurrence. This speaks in favor of my contention that the starting point of gangosa is the skin yaw, either initial yaw or late ulcerating yaws lesion, extending directly into the mucous membrane. The initial yaw lingers there for some time after the corresponding early skin lesion has healed and the allergic state of the body organism which is responsible for the deep ulcerating manifestation of yaws has been reached. The late ulcerative yaws lesion of the skin directly penetrates the roof of the nose. The statement of Plehn and Mense, junior,(6) is at divergence with this contention. These authors claim that frambœsic lesions on the faces of gangotic patients are "eminently rare;" yet they present photographs of eight cases of tertiary yaws, including four of early and advanced gangosa. These photographs are reproduced from the excellent work of Hallenberger.(11) Every one of the gangosa cases shows unmistakable manifestations of yaws on the skin of the face, mostly on the skin of the nose. The photograph of an

advanced case (11, fig. 46) of gangosa in man shows a condition practically identical with that in our case, the monkey shown in Plate 24, fig. 1. While two others (11, figs. 4 and 44) are identical with the lesions in the monkey shown in Plate 22, fig. 2, as far as the changes on the nose are concerned.

The paper of Dijke, Bakker, and Hoesen(7) bears on this question. All of their nine cases of gangosa showed frambœsic lesions on the face, a good many of them about the nose.

I strongly suspect that the papule or tubercle designated as the initial lesion in 1891 by Numa Rat,(24) and mentioned by him as sometimes observed on the palate as a primary gangotic lesion, was really an extension of a much older, if not more intensive, nasal lesion that had remained unnoticed or healed up to that time. That the ulcers on the palate described by Leys(14) and by Mink and McLean(18) had nothing to do with gangosa needs no further discussion.

It is remarkable that this interpretation of the pathogeny of gangosa has been handed down in the literature in a uniformly worded definition in very much the same way as has the erroneous teaching that the primary lesion of yaws is an ulcer. The observations on experimentally produced yaws of Hallenberger(11) in Africa, and of Sellards, Lacy, and Schöbl(30) in the Philippines, prove that the initial uncomplicated frambœsic lesion is a yaw and not an ulcer. Naturally, in a clinic, the intact initial mother yaw is rarely seen, and the very earliest stage of gangosa probably never.

I have repeatedly observed that the initial frambœsic lesion in monkeys enters the mucocutaneous border of the nose (see Plate 15), and remains there for some time after the skin lesions have disappeared. In some of these animals gangosa developed from the residual initial lesion on the nasal mucous membrane upon superinfection. Hallenberger (11, fig. 4) describes and illustrates a similar case in man, where metastatic early yaws of the skin extended to the nasal mucous membrane, and he remarks that he never saw a primary yaws lesion on a mucous membrane. In his observations on yaws of the genitalia he noted that the lesions extended from the skin onto the mucous membrane of the vulva. Hallenberger(11) also observed many cases of rhinopharyngitis mutilans among the mountain tribes in Kameroon. All of his cases "have gone thru Mabatta (yaws) and still showed on the outer nose frambœsic efflorescences which stood in connection with the pathologic process in the inner

nose." According to Hallenberger(11) the cutaneous portion of the overlapping lesion yields more quickly to treatment than does that portion involving the mucous membrane. I have observed the same phenomenon in experimental animals during spontaneous healing. The fact that gangosa has been experimentally produced in animals in more than one instance by direct extension of skin lesions, either early or late, onto the mucous membrane of the nose shows that this mode of pathogeny is the commonest if not the only one.

NODULAR LUPUSLIKE LATE FORMS OF YAWS

In certain instances the experimental initial yaw persists for a long time and migrates over the forehead or scrotum, as the case may be. It does not heal by complete restitution, as is the case in early lesions, but by scars that branch out and spread over the entire affected area. The skin becomes pigmented, thin, and glossy, and over this scarred area islets of flat defects or elevated nodules covered with scabs are scattered. The entire lesion assumes an appearance of lupus vulgaris and simulates very closely late yaws lesions in man that have been designated as lupuslike or late nodular yaws lesions.(9) An illustration of this type of lesion as it occurred on the forehead of a gangotic monkey is given in Plate 22, fig. 1.

LATE PERSISTENT FRAMBŒSIDES

KERATODERMA PLANTARE AND ICHTHYOTIC DESQUAMATIVE SKIN LESIONS

In their discussion of the pathogeny of frambœsia Castellani and Chalmers(5) make the following statement:

> There appears to be a quarternary stage, as yet little recognized, in which the organism cannot be found, in which, however, it has so chemically sensitized the cells that, upon stimulation by as yet little known agents, lesions appear which seem to be of a para-anaphylactic origin e. g. KERATODERMA.

The callous thickening of the plantar skin as well as the ichthyotic skin lesions found on the dorsum pedis, which were extensively described and illustrated by Baermann(2) in the Dutch Indies, and by Hallenberger(11) in Kameroon, have been associated with frambœsia as its late manifestation. That this condition exists in the Philippines follows from the illustrated publications of P. D. Gutierrez.(9) Their etiologic affiliation to yaws rests on purely clinical grounds. Treponemas have never

been found in those lesions and probably never will be; at any rate, if present at all, the finding alone of one or two treponemas cannot explain the extensive hypertrophic and cornification process. Experimental production of these dermatoses in frambœsic animals is therefore of great significance and proves conclusively their etiology.

The main feature of the keratoderma plantare in monkeys was that the lesions developed very slowly and were of long duration. They consisted in a horny thickening, first of that part of the skin that covers the heel. Later other protuberances of the sole became similarly affected. The cornification of the epidermis was most pronounced on the heel throughout the disease. The callous pads were thickest in the center and gradually, without interruption, vanished into the soft skin of the instep, which at first remained normal. The surface of the callosities was, generally speaking, smooth at first; the diffuse, minute loosening of the most superficial layers of the hypertrophic epidermis was responsible for the slate grayish or whitish color of the surface. These lesions represented from the beginning a purely hypertrophic process, without any inflammatory reaction whatever. Being symmetric and of the same extent on both soles, they were restricted to the plantæ pedum. The palms remained normal throughout.

As the disease progressed the thickening of the affected parts increased, the surface became uneven, and exfoliation supervened in the form of shallow, round defects with exfoliated edges. By confluence, these defects became maplike with a cribrated basis. The affected areas remained stationary, with the exception of the outer plantar margin. The shallow, tough exfoliation was most pronounced at this site, as it was at the margin of the heel. Gradually, the lesion crossed the outer dorsoplantar border and spread over onto the skin of the outer part of the dorsum pedis, and at the same time assumed a change in character. The skin of the dorsum adjacent to the dorsoplantar border and over the metatarsal region clear to the dorsal surface of the toes became ichthyotic. On the parchment-like, slightly scaly skin appeared numerous isolated, circular efflorescences, measuring from 2 to 5 millimeters in diameter. They were elevated, dry, concentric lesions showing very little, if any, marginal exfoliation. The soft skin of the insteps became similarly affected. To all appearances the last-described ichthyotic lesions are identical with those seen on the same part of the body in late frambœsia in man. They are truer

to the illustration of patients given in the literature (for instance, by Baermann,(2) Gutierrez,(9) and others) than are similar but topographically unique late frambœsides on the monkey's tail, though identical in character and nature, due evidently to a similar topographic condition of the skin.

Keratoderma plantare and the peculiar late frambœsic ichthyotic exfoliative skin lesions on the dorsum pedis have been experimentally produced in animals by superinfection with yaws. The etiology of these lesions no longer rests on clinical observations alone, in as much as, in the absence of demonstrable treponemas in these lesions, the only possible proof of their etiology has been given, a proof more valid than the demonstration of occasional treponema could furnish. The pathogenesis of the keratoderma in particular has been explained. This condition does not begin as metastatic yaws modified by the anatomic conditions existing in the sole of the foot, but begins, from the very start, as keratoderma. The keratoderma plantare and the ichthyotic desquamative dermatoses affecting the dorsum pedis are late persisting frambœsides. They are one and the same process, the difference in their clinical manifestation being due to anatomical conditions of the skin of the dorsum pedis different from those on the sole of the foot. The photographs in Plate 23, fig. 2, and Plate 24, fig. 2, picture a well-developed, uncomplicated keratoderma plantare in the monkey. The pus-secreting fissures and ulcers met with in some patients afflicted with this condition are undoubtedly the result of traumatic injuries and consequent invasion of extraneous matter, including bacteria. Interesting is the fact that, as in most cases in man, this disease developed in monkeys on the lower extremities only where, in spite of the use of all four extremities in locomotion, the bulk of the body weight rests. May not the constant pressure bring forth the tendency of yaws toward the hypertrophy and cornification of epidermis, so manifest in other forms of frambœsia, to its maximal expression on the sole of the foot?

As a consequence of specific treatment, the experimental keratoderma plantare healed in reverse order to that in which it developed; so that in a certain stage of regression the early stages of its development were simulated. Within four days after the first intramuscular injection of neosalvarsan the healing, first, of the lesion on the dorsum pedis and on the soft skin of the instep was marked. The hypertrophic and cornification process became limited to the foot pads, and even these gradually regressed on further treatment.

Psoriasis palmaris frambœsica must be strictly differentiated from the condition just described. Whether it occurs on the soles of the feet or on the palms of the hands, the condition known as keratoderma is a late manifestation of yaws. It is a progressive diffuse hypertrophic and cornifying process of the skin with exfoliation. Healing does not occur during the active stage.

Psoriasis palmaris frambœsica, on the other hand, is an early general frambœsic lesion, manifested by flat elevated macules with a slightly inflammatory basis, covered with exfoliating epidermis. It consists of separated lesions of various ages. While some are healing, others make their appearance.

Comparing the experimental palmar and plantar manifestations of yaws in monkeys with the lesions commonly encountered in patients, it must be remembered that there are at least three common plantar and palmar yaws lesions in man. First, is the more or less typical metastatic yaw on the sole of the foot that may be somewhat modified when it reaches the neighborhood of the foot pads; second, there is the fairly common psoriasis palmaris which has little resemblance to the typical yaw, being a macular lesion with a reddish elevated basis and shallow and concentric circular desquamation. There is no oozing and no formation of crust. This form is indistinguishable from psoriasis palmaris syphilitica; the third common manifestation of frambœsia on the palms, but more frequently on the soles of the feet, is the keratoderma. There is still less resemblance between the last-mentioned lesion and the typical yaw, it being a diffuse thickening of the skin with secondary desquamation characterized mostly by cornification of the epidermis, pronounced acanthosis, and very slight inflammatory reaction on the part of the cutis. While the first-mentioned two lesions are coincident with the metastatic crops of typical yaws, the third one is a late manifestation and is persistent.

Either of the three yaws lesions, when located on the sole of the foot, is termed "clavos" by the patients.

The coincidence of the first or the second lesion with the keratoderma may create the impression that the basic lesion of keratoderma is a metastatic yaw more or less modified. Experimental evidence, however, shows that this is not the case. In monkeys that have been kept constantly infected by superinfection I have repeatedly seen keratoderma plantare develop from the very beginning as a hypertrophic, diffuse, persistent

process that responded promptly, even though slowly, to specific treatment with neosalvarsan. On the other hand, the experimental psoriasis palmaris described above was accompanied by a general eruption of typical and ringworm yaws in other parts of the body. Induration and slight desquamation without oozing and without the formation of crusts, was typical of this lesion in monkeys. The process was evanescent and typical of psoriasis frambœsica in man.

Keratoderma plantare was noted in monkeys that had been repeatedly superinfected. Some of these animals had metastatic early yaws lesions, while others failed to develop them. Psoriasis palmaris developed only in animals that presented early generalized yaws.

I have successfully produced the initial local yaw, the typical metastatic, the ringworm form, the papular yaws, the early evanescent, the late persistent frambœsides, nodular or lupuslike forms, and the ulcerative lesions of late yaws, including gangosa, in Philippine monkeys.

NONSPECIFIC MANIFESTATIONS OF YAWS IN PHILIPPINE MONKEYS

In enumerating the various symptoms I wish to mention marasmus, alopecia, hyperpigmentation, leucoderma, and lymphadenitis.

After the animals had been kept infected for a certain length of time the nonspecific changes appeared. General atrophy and more or less pronounced diffuse alopecia were noticeable. These gave the animal a peculiar appearance not unlike that of newborn syphilitic babies (Plate 27, fig. 1).

Although the animals were apparently normal and took food regularly, the marasmus in certain instances progressed. Due to this general condition the mortality of the infected animals was rather high and only a few of them survived more than two years after infection had commenced. The usual postmortem finding in animals that died in the later part of frambœsic infection was that of general marasmus—that is, absence of fatty tissue, atrophy of the liver and heart, and serous clear transudation into the peritoneum, pleura and, most commonly, into the pericardial sack. The spleen was invariably small and firm and no signs were found of bacterial infection except in one case where, in an extremely marantic monkey, typical pneumonia was found corresponding in every respect to the findings made in our experimental work on pneumonia in Philippine monkeys under natural climatic conditions.(28)

In the description of the initial local yaw mention was made of a temporary loss of hair within the area of the monkey's skin affected by the lesion. As the lesion spreads on the periphery and heals in the center the hair is usually restored. Aside from this local alopecia a generalized alopecia is not infrequently found in animals that have been infected for several months. It begins as a rule in the parietal regions. It is a diffuse, not patchy alopecia, and is symmetric. It is frequently noticed in the face and may affect also the extremities. This happens, not by direct extension of the alopecia from the head over the neck to the arms, but on the extremities it usually commences on the distal ends of the feet and the hands, and spreads upward to about the middle of the forearm. In these places the alopecia is most pronounced and displays particular predilection for the tail. It may, however, to a more or less pronounced degree affect the entire body. This generalized diffuse alopecia is persistent, but the hair is sooner or later restored following specific treatment. This phenomenon of lack of hair was observed before on Philippine monkeys, by Ashburn and Craig.(1) The condition, however, may occur in noninfected marantic monkeys, although not so frequently as in yaws animals.

In their attempt and with the intention to accumulate as many differential points as possible between frambœsia tropica and syphilis, frambœsologists, particularly those working in Africa, stated that, while alopecia is very common with syphilis, it is unknown to be a symptom of frambœsia. One naturally wonders as to the validity of this statement if one considers the absence of alopecia of any kind among pure Africans. My experiments confirm the observation of Ashburn and Craig(1) and further support the point that, with respect to alopecia, there is no innate difference in the nature of *Treponema pertenue* and *T. pallidum.*

Hyperpigmentation of the skin is one of the characteristics of yaws in monkeys. It is not so pronounced on the eyebrows and face as it is on the other parts of the body. It is a discolorization which is restricted to the area of healed yaws, either local or metastatic, and persists a long time. It is particularly pronounced on the scrotum.

Aside from the hyperpigmentation of the skin hyperpigmentation of the hair was noticed in several instances over the eyebrows and on the skull. When the spreading initial local yaw reached its climax and became stationary there was noticed

a deep black discolorization of the hair outlining the healed lesion as a narrow black line over the eyebrows or on the top of the skull. It persisted for some time after the lesion itself had healed but disappeared ultimately without treatment.

The lack of pigment, or leukoderma, can be best seen in the face round the eyebrows and on the bridge of the nose, where a sharp dividing line naturally separates the pigmented parts of the entire body from the rather whitish skin on the eyelids. In a great many instances this line was found interrupted and leukodermic patches overlapping across the line into the pigmented part of the skin were observed (see Plate 17, fig. 1). The leukodermic patches, as a consequence of yaws lesions, were still more persistent than the hyperpigmentation.

THE RELATION BETWEEN THE TYPE AND CHARACTER OF EXPERIMENTAL YAWS LESION AND THE NUMBER OF TREPONEMAS ENCOUNTERED THEREIN

The number of treponemas found by the dark field in an experimental yaws lesion is variable. The number of treponemas that will be found on microscopic examination can, however, be safely predicted from the appearance or type of the lesion.

As to the type of the lesion, the general rule is that initial and metastatic lesions contain regularly treponemas in sufficient numbers to be demonstrated easily under the dark field. The late lesions, however, either ulcerative, lupuslike, or nodular, are characterized by the presence of so few treponemas that even a persistent and repeated search fails to detect them.

As for the frambœsides, it has already been mentioned that the search in them for *Treponema pertenue* was futile.

Aside from the type of the lesion, the initial local yaw, the metastatic generalized yaw and the late manifestations of yaws, the character of a local or of a metastatic early yaw indicates with fair constancy the number of spirochætes it can be expected will be found upon microscopic examination of the oozing lymph or of the scrapings from the lesion. The elevated, freely oozing, extensive lesion contains very numerous treponemas, while the feeble, dry lesion contains very few. When the healthy, freely oozing lesion, containing numerous treponemas, reaches the third or fourth stage of development and becomes dry and spreads on the periphery, it shows the presence of but few treponemas, while in the last two stages in the development of a local yaw there are, as a rule, none.

In spite of the apparent absence of treponemas, the scrapings from the lesion may produce a take of typical yaw, but this procedure is not reliable and gives occasional failures of takes upon careful inoculation of normal animals. Care should, therefore, be exercised in the selection of material for reinoculation or superinfection, particularly when a test of resistance or immunity comes into consideration.

Repeatedly a long search for treponemas in lymph glands, for instance, failed to reveal the presence of treponemas under the dark field, while inoculation of the suspension of the entire gland produced in the monkey a typical local lesion in which the treponemas were demonstrated microscopically with ease. Thus, in frambœsia the inoculation is a more reliable procedure than is the microscopic search for treponemas.

TERMINOLOGY OF THE VARIOUS FORMS OF YAWS

The classification of yaws into primary, secondary, and tertiary stages has been made by analogy with the familiar terminology in syphilis. This classification necessarily suggests a certain sequence of appearance of the lesions and even a certain dependency of the secondary lesion on the primary and of the tertiary on the secondary, as well as latency of infection separating the three stages from each other. That this classification is not correct, at least with respect to yaws, has been pointed out in the literature.

In our observations(30) on experimental yaws in humans the fact was established by experimentation that the typical initial yaw need not develop at all in man, and yet typical, metastatic, generalized yaws will follow (frambœsia d'embleè). Consequently, the frequent clinical experience, that the primary yaw cannot be discovered by questioning the patient, need not be altogether due to the patient's ignorance. As in man, so in the monkey, there is not necessarily a period of latency between the initial, local lesion and the generalized manifestations of yaws.

As in man, so in monkeys, the initial yaw may and usually does survive the metastatic yaws. On the other hand, I have observed that, following the persistence of the initial lesion produced by superinfection, the animal's organism reached a state in which the subsequent superinfection no longer produced

a typical yaw but, instead, produced a deep, ulcerating, slowly growing lesion that did not heal while active, and never from the center affected the entire thickness of the skin, the subcutaneous tissue, and cartilages, and ultimately healed from the periphery by deep scars; in other words, a lesion usually termed tertiary. Therefore, lesions in these cases of experimental yaws, designated usually as secondary, never took place and the infection in the animal passed from the stage termed as primary directly into the tertiary. It is at once evident from this that the systematic classification of yaws, either in man or in the animal under experimentation, into primary, secondary, and tertiary stages is untenable. I have, indeed, seen in an individual, superinfected animal, the direct transition of the so-called primary lesion into a so-called tertiary lesion, the animal, in the meantime, not having passed through either a stage of latency or through the so-called secondary stage.

Deep, late ulcerative lesions do not develop metastatically as such. They spring from early yaws of long duration. The character of these lesions, so different from the early superficial manifestations, is due to the allergic state of the affected animals or of man. One frequently sees in the literature photographs of lesions of a deep ulcerative character in man, that have been labeled "primary lesion," apparently for the sole reason that they developed at the site of the mother jaw. If the division into primary, secondary, and tertiary stages is to be insisted upon, the paradoxical designation of such a lesion as "primary tertiary" would be justified; primary because original or initial yaw, and tertiary because of its clinical and anatomical character. It, therefore, would be more literal and less misleading to designate the yaws lesions as suggested in the following paragraphs.

The term of the initial lesion as a mother yaw is acceptable in view of the fact that the generalized eruption of typical yaws occurs in man most frequently. The designation of this lesion as an initial local yaw would, however, at the same time, include the initial local yaw that develops without becoming a mother yaw to a subsequent generalized eruption.

The generalized eruption of typical yaws should be designated as a metastatic yaw, typical or atypical.

For the form of superficial metastatic lesions of protean character that develop toward the end or subsequent to the cropping

out of typical yaws, the name of frambœsides should be retained. However, besides the designation of early and late frambœsides, as is suggested by Hallenberger,(11) some should be called evanescent and others persistent frambœsides. While the evanescent frambœsides, which are in a given case the early ones, still maintain some of the resemblance to certain stages in development of the initial local yaw; the persistent ones, which are the late frambœsides, have little if any resemblance to the local, and still less to the metastatic yaw. In them the hyperkeratosis, hyperpigmentation, and depigmentation are pushed into the foreground.

The lesions that are generally termed tertiary should be termed late yaws. Further determination of these lesions should be made by descriptive terms. Whether they produce mutilation or not is largely dependent on the shrinking scars by which they heal, on the extent of the ulceration, or on their topographic location (gangosa).

As a suggestion we would propose the following terms with regard to types of yaws lesions:

1. Local, initial yaw, typical or atypical.
2. Metastatic yaw, regionary or generalized, typical or atypical.
3. Local or metastatic, evanescent or persistent frambœsides.
4. Late yaws lesions, ulcerative, nodular, and lupuslike.
5. The bone lesions should be classed by themselves, in as much as they are either early or late, hypertrophic, atrophic, or necrotic. They may affect the periost or the bone itself.

Disregarding the type of the lesion or its genesis, the yaws infection could be divided into two stages; namely, first, the stage during which the patient is susceptible to superinfection after or before he has been cured, and, second, the stage in which the patient cannot be superinfected, either before or after treatment. This division would be of particular importance from the epidemiological standpoint. It may be safely predicted that a person, who has had yaws for about eight months or a year and gone through typical crops of metastatic yaws, will no longer contract yaws, either naturally or experimentally. However, patients that have had yaws for not longer than about a half year may again contract typical yaws infection. The cases of late ulcerative and hypertrophic yaws manifestations represent a protracted first stage.

SEROLOGIC OBSERVATIONS ON EXPERIMENTAL YAWS IN PHILIPPINE MONKEYS [9]

The purpose of the serologic investigation was to ascertain the following points:

Serologic findings in—
- Experimental initial local yaw.
- Experimental generalized yaws.
- Experimental ulcerative forms of yaws.

The influence on serologic findings of—
- Superinfection.
- Treatment.
- Reinoculation.

First of all, an attempt was made to learn the frequency, regularity, and other conditions of Wassermann reaction in the early stage of a noncomplicated initial, local yaw. For this purpose a series of normal monkeys was subjected to the Wassermann reaction, after which the animals were inoculated with yaws. In Tables 2 and 3, showing the development and duration of the Wassermann reaction in singly infected monkeys that developed an initial, local yaw the results of the Wassermann reaction are given. It can be seen that approximately one month after the inoculation and about the time of the appearance of the clinical lesion recognizable as yaw the positive Wassermann reaction appeared. However, at least two out of eight successfully inoculated animals showed persistently negative Wassermann reaction. On the other hand, those animals that failed to take (two monkeys) gave persistently negative Wassermann reaction. It can be further seen from these tables that the duration of the positive reaction in the majority of the cases was very short, only one having persisted distinctly positive for more than three months after inoculation and for two months after the appearance of the clinical lesion. The majority of positive reactions became distinctly weaker and most of them were negative in from five to six weeks after inoculation. This peculiar behavior as to the appearance and short duration of Wassermann reaction in the initial uncomplicated local yaw should not be wondered at, because an analogous condition hardly ever occurs in man, where practically in every case the initial

[9] Onofre Garcia and Jose Ramirez, of the division of biology and serum laboratory of the Bureau of Science, collaborated in preparing this section, pages 261 to 276.

TABLE 2.—*Showing the development and duration of positive Wassermann reaction in singly infected monkeys that developed initial local yaw.*

[—, complete hæmolysis; ±, trace of inhibition; +, slight inhibition; ++, about 50 per cent inhibition; +++, about 75 per cent inhibition; ++++, 100 per cent inhibition; 0, not done; 00, failure to take.]

Designation of monkey.	First inoculation.	First appearance of clinical lesion.	Date and results of successive Wassermann reactions.									
			First examination.	Wassermann reaction.	Second examination.	Wassermann reaction.	Third examination.	Wassermann reaction.	Fourth examination.	Wassermann reaction.	Fifth examination.	Wassermann reaction.
H-20	a XI- 8-26	XII- 7-26	XI- 9-26	—	XI- 6-26	—	XII- 2-26	—	XII- 9-26	—	XII-16-26	—
J-15	XI- 5-26	XII- 2-26	do	—	do	—	do	±	do	+	do	—
J-16	do	do	do	—	do	—	do	±	do	±	do	—
A-5	X-22-26	XI-24-26	XI-17-26	—	XII- 2-26	+	do	+	do	++	do	+
N-11	XI-18-26	XII- 2-26	XI-19-26	—	do	—	do	0	do	+	do	±
M-5	XI-12-26	00	do	—	do	—	do	0	do	—	do	—
L-6	XI-15-26	00	do	—	0	0	do	0	do	—	do	—
K-6	XI-17-26	XII- 2-26	do	—	XII- 2-26	±	do	0	do	±	do	—
G-8	XI-16-26	XII-14-26	do	—	do	—	do	0	do	—	do	—
E-16	X-27-26	XII- 7-26	do	—	do	—	do	0	do	±	do	—

Designation of monkey.	Date and results of successive Wassermann reactions.		Date of superinfection.	Date and results of successive Wassermann reactions.									
	Sixth examination.	Wassermann reaction.		Seventh examination.	Wassermann reaction.	Eighth examination.	Wassermann reaction.	Ninth examination.	Wassermann reaction.	Tenth examination.	Wassermann reaction.	Eleventh examination.	Wassermann reaction.
H-20	XII-23-26	—	XII-27-26	I- 5-27	±	I-15-27	±	I-27-27	±	II-15-27	—	II-28-27	±
J-15	do	—	do	do	—	do	±	do	±	do	±	do	—
J-16	do	—	do	do	0	do	0	do	0	do	0	do	0
A-5	do	+	do	do	+	do	±	do	±	do	++	do	—
N-11	do	—	do	do	—	do	—	do	±	do	0	do	0
M-5	do	—	do	do	—	do	—	do	±	do	—	do	—
L-6	do	—	do	do	—	do	—	do	++++	do	+++	do	—
K-6	do	—	do	do	—	do	—	do	++	do	—	do	—
G-8	do	—	do	do	0	do	0	do	0	do	0	do	0
E-16	do	—	do	do	0	do	0	do	0	do	0	do	0

a These letters and figures indicate month, day, and year; thus, XI-8-26 means November 8, 1926.

yaw is followed either by generalization of the yaws process or by chronic lesion that persists for a long time. In animals, on the contrary, in the majority of cases the local yaw develops, regresses, and heals, by which process the entire infection is terminated. For that reason there are cases where the local lesion during its development and regression, and following its healing, brings about no appreciable change in the serologic behavior of the animal's serum. In order that the reasons for such irregularities as occur in the Wassermann reaction in experimental yaws might be detected, animals in various stages of yaws infection were examined. They represented all of the modifications in the arrangement of infection, superinfection, and reinfection that we applied in our investigation and the results are given in Tables 4 to 8.

The basic table giving the results of the Wassermann test performed on yaws-infected monkeys from time to time has been arranged in Table 4 according to the time factor. It shows in months the time that elapsed between the first inoculation and the date on which the sample of blood was withdrawn from the heart of the infected animal for use in the Wassermann reaction. From this general table one conclusion only can be drawn; that is, the effect of duration of infection upon strength or development of the positive Wassermann reaction. It can be noted that, following the fourth month, strong Wassermann reactions make their appearance.

In Table 7 the data with regard to the result of the Wassermann reaction in yaws-infected monkeys have been arranged, and five factors are considered.

TABLE 3.—*Showing the rapid disappearance of Wassermann reaction in experimental local yaw of short duration (one inoculation) upon spontaneous healing.*

[—, complete hæmolysis; ±, trace of inhibition; ++, about 50 per cent inhibition; +++, about 75 per cent inhibition.]

Designation of monkey.	Date of first inoculation.	Date of appearance of lesion.	Date of examination, Wassermann reaction.	Result.	Date of reëxamination upon healing.	Result.
T-9	VIII-18-26	IX-6-26	X-6-26	+++	XI-4-26	—
J-14	IX-30-26	X-9-26	----do----	±	----do----	—
M-4	IX-13-26	-----do-----	----do----	±	----do----	—
B-4	VIII-6-26	IX-22-26	----do----	+++	----do----	—
P-11	VI-25-26	VII-16-26	----do----	++	----do----	—
K-5	IX-23-26	X-9-26	----do----	—	----do----	—
H-19	IX-29-26	-----do-----	----do----	±	----do----	—
T-10	VIII-20-26	IX-6-26	----do----	+++	----do----	—

First is the factor of the number of successive inoculations. This factor was considered with the view to obtaining some information, if possible, as to whether or not the number of successive crops has any influence on the development of the Wassermann reaction in yaws monkeys.

The second factor, column 2, is the number of simultaneous inoculations; that is, in one infection the inoculation was sometimes carried out at one place only, at other times in two places, and at still other times in three places, so that an animal may

TABLE 4.—*Showing the influence of number of takes upon the result of the Wassermann reaction.*

[—, complete hæmolysis; ±, trace of inhibition; +, slight inhibition; ++, about 50 per cent inhibition; +++, about 75 per cent inhibition; ++++, 100 per cent inhibition.]

Monkey No.	Takes.	Wassermann reaction.	Months.	Form of yaws.
O–a	0	—	1	Local.
M–2	4	+	1	Do.
M–3	5	+	1	Do.
T–1	1	—	1	Do.
S–b	3	±	3	Do.
F–11	2	—	3	Do.
O–b	2	—	3	Local, mild.
P–9	0	—	3	Local.
P–10	4	++	3	Do.
D–9	1	—	3	Do.
B–3	3	++	4	Do.
C–1	2	—	4	Ulcerative.
H–16–b	2	+++	5	Local intensive exacerbation.
P–8	3	++	6	Generalized.
D–8	2	±	6	Local.
J–10	2	—	6	Ulcerative.
D–9	1	++	6	Local.
S–b	3	++	6	Do.
B–3	3	++++	6	Do.
C–1	2	++	6	Ulcerative.
B–1	[a]3	—	7	Local.
B–2	2	+++	7	Do.
O–b	2	—	8	Do.
D–8	2	±	8	Do.
J–10	2	+	8	Ulcerative.
P–8	3	++	9	Generalized.
B–1	3	+	9	Local.
B–2	2	++++	9	Local, extensive.
A–2	4	++	9	Local.
A–3	2	—	9	Do.
L–4	2	++	9	Do.
A–2	4	+++	11	Do.
A–3	2	+	11	Do.
L–4	2	++	11	Do.

[a] One mild take.

have been infected only once but in one place, in two places, or in three places. This factor is given here regardless of whether the individual inoculation resulted in a take or not.

The third factor is the number of takes; that is, the total number of takes that developed following any number of inoculations made at any number of successive infections.

The fourth factor is the one considered in the first table; that is, the factor of duration of infection.

The fifth factor, in the last column, is the form of the lesion that developed. General classification is made into local typical

TABLE 5.—*Showing the influence of number of infections upon the result of the Wassermann reaction.*

[—, complete hæmolysis; ±, trace of inhibition; +, slight inhibition; ++, about 50 per cent inhibition; +++, about 75 per cent inhibition; ++++, 100 per cent inhibition.]

Monkey No.	Infections.	Wassermann reaction.	Months.	Form of yaws.
O–a	1	—	1	Local.
M–2	1	+	1	Do.
M–3	1	+	1	Do.
T–1	1	—	1	Do.
S–b	1	±	3	Do.
F–11	1	—	3	Do.
O–b	2	—	3	Local, mild.
P–9	1	—	3	Local.
P–10	1	++	3	Do.
D–9	1	—	3	Do.
B–3	3	++	4	Do.
C–1	2	—	4	Ulcerative.
H–16–b	1	+++	5	Local intensive exacerbation.
P–8	2	++	6	Generalized.
D–8	1	±	6	Local.
J–10	2	—	6	Ulcerative.
D–9	1	++	6	Local.
S–b	2	++	6	Do.
B–3	3	++++	6	Do.
C–1	2	++	6	Ulcerative.
B–1	3	—	7	Local.
B–2	3	+++	7	Do.
O–b	3	—	8	Do.
D–8	1	±	8	Do.
J–10	2	+	8	Ulcerative.
P–8	2	++	9	Generalized.
B–1	3	+	9	Local.
B–2	3	++++	9	Local, extensive.
A–2	2	++	9	Local.
A–3	2	—	9	Do.
L–4	1	++	9	Do.
A–2	3	++	11	Do.
A–3	2	+	11	Do.
L–4	1	++	11	Do.

yaw, extensive exacerbation or extensive lesion, mild lesion, generalized yaws, and ulcerative type.

The results in experimental animals have been arranged in this table, according to the duration of infection, into ten groups. Although some of the groups are rather small, the others are sufficiently large to draw, by comparison, certain conclusions with regard to one or the other factor considered in some of the small groups.

If we consider the first group, the one-month group, we can see that the number of successive infections, the time factor, and the form of yaws are constant. There are, however, va-

TABLE 6.—*Showing the influence of duration of yaws infection upon the result of the Wassermann reaction.*

[—, complete hæmolysis; ±, trace of inhibition; +, slight inhibition; ++, about 50 per cent inhibition; +++, about 75 per cent inhibition; ++++, 100 per cent inhibition.]

Monkey No.	Months.										
	1	2	3	4	5	6	7	8	9	10	11
O–a	—		±								
M–2	+										
M–3	+										
T–1	—										
T–9	±										
T–10	±										
A–4	—										
E–15		—									
B–4		±									
J–13		±									
T–4		—									
K–4		—									
S–b			±			++					
F–11			—								
O–b			—					—			
P–9			—								
P–10			++								
D–9			—			++					
P–11			—								
B–3				++		++++					
C–1				—		++					
H–16–b					+++						
T–3					±						
N–8					++++						
P–8						++			++		
D–8						±		±			
J–10						—		±			
C–3						++++					
B–1							—		+		
B–2							+++		++++		
A–2									++		+++
A–3									—		+
L–4									++		++

TABLE 7.—*Combined table, showing the influence of severity of lesion, duration of infection, repeated inoculations, number of inoculations, number of takes, and type of yaws upon the Wassermann reaction.*

[—, complete hæmolysis; ±, trace of inhibition; +, slight inhibition; ++, about 50 per cent inhibition; +++, about 75 per cent inhibition; ++++, 100 per cent inhibition.]

Monkey No.	Successive infections.	Individual inoculations.	Positive takes.	Wassermann reaction.	Duration of infection.	Form of yaws.
					Months.	
O-a	1	2	0	—	1	Local.
T-1	1	3	1	—	1	Do.
A-4	1	2		—	1	Do.
T-9	1	1	1	±	1	Do.
T-10	1	2		±	1	Do.
M-2	1	5	4	+	1	Do.
M-3	1	5	5	+	1	Do.
E-15	2	2	2	—	2	Do.
B-4	1	1	1	±	2	Do.
J-13	2	2	2	±	2	Do.
T-4	2	2	2	—	2	Do.
K-4	1	2		—	2	Do.
P-9	1	8	0	—	3	Do.
P-11	1	1	1	—	3	Do.
D-9	1	2	1	—	3	Do.
F-11	1	2	2	—	3	Do.
O-b	2	2	2	—	3	Do.
S-b	1	6	3	±	3	Do.
P-10	1	7	4	++	3	Do.
C-1	2	2	2	—	4	Ulcerative.
B-3	3	7	3	++	4	Local, extensive.
T-3	7	7	0	—	5	
H-16-b	1	2	2	+++	5	Local, intensive exacerbation.
D-8	1	2	2	±	6	Local.
J-10	2	2	2	—	6	Ulcerative.
D-9	1	2	1	++	6	Local.
S-b	2	6	3	++	6	Do.
B-3	3	7	3	++++	6	Local, extensive.
C-1	2	2	2	++	6	Ulcerative.
C-3	2	3	3	++++	6	Generalized.
P-8	2	3	3	++	6	Do.
B-1	3	3	2	—	7	Local, mild.
B-2	3	5	3	+++	7	Local, extensive.
O-b	3	5	2	—	8	Local.
D-8	1	2	2	±	8	Do.
J-10	2	2	2	+	8	Ulcerative.
A-3	2	4	2	—	9	Local.
B-1	3	3	3	+	9	Do.
L-4	1	2	2	++	9	Do.
A-2	2	6	4	++	9	Do.
P-8	2	3	3	++	9	Generalized.
B-2	3	6	2	++++	9	Local, extensive.
A-3	2	4	2	+	11	Local.
L-4	1	2	2	++	11	Do.
A-2	3	8	4	+++	11	Do.

riations in the number of inoculations and the number of takes. There are also variations in the results of the Wassermann reaction. There appeared to be a relation between the strength of the Wassermann reaction and the number of inoculations, and the number of takes. The two frankly positive Wassermann reactions were found in monkeys that had been inoculated in five places and developed four and five takes, respectively. It seems to show that these two factors are significant; but, whether both of them or only one is so, and which one, is not apparent from this group.

TABLE 8.—*Showing the influence of number of inoculations upon the result of the Wassermann reaction.*

[—, complete hæmolysis; ±, trace of inhibition; +, slight inhibition; ++, about 50 per cent inhibition; +++, about 75 per cent inhibition; ++++, 100 per cent inhibition.]

Monkey No.	Inoculations.	Wassermann reaction.	Months.	Form of yaws.
O–a	2	—	1	Local.
M–2	5	+	1	Do.
M–3	5	+	1	Do.
T–1	3	—	1	Do.
S–b	6	±	3	Do.
F–11	2	—	3	Do.
O–b	2	—	3	Do.
P–9	8	—	3	Do.
P–10	7	++	3	Do.
D–9	2	—	3	Do.
B–3	7	++	4	Do.
C–1	2	—	4	Ulcerative.
H–16–b	2	+++	5	Local, intensive exacerbation.
P–8	3	++	6	Generalized.
D–8	2	±	6	Local.
J–10	2	—	6	Ulcerative.
D–8	2	++	6	Local.
S–b	6	++	6	Do.
B–3	7	++++	6	Do.
C–1	2	++	6	Ulcerative.
B–1	3	—	7	Local.
B–2	5	+++	7	Do.
O–b	5	—	8	Do.
D–8	2	±	8	Do.
J–10	2	+	8	Ulcerative.
P–8	3	++	9	Generalized.
B–1	3	+	9	Local.
B–2	6	++++	9	Local, extensive.
A–2	6	++	9	Local.
A–3	4	—	9	Do.
L–4	2	++	9	Do.
A–2	8	+++	11	Do.
A–3	4	+	11	Do.
L–4	2	++	11	Do.

In the second group, the two-month group, the factors of time and form of lesions are the same. The factors of number of successive infections, the number of inoculations, and the number of takes do not vary considerably, the lowest being one and the highest two. In consequence, the results of the Wassermann reaction do not vary either, there being three negative and two doubtful Wassermann reactions in this group.

In the three-month group the factor of number of successive infections does not vary much, only one animal having had two successive infections; the rest of them were infected only once. The time factor does not vary the number of individual inoculations, however, and the factor of takes varies. The factor of form of yaws is slightly modified in one animal that developed a mild lesion after inoculation. In this group, better than in other groups, the predominance of the factor of takes over that of individual inoculations is apparent. The animal that developed the highest number of takes showed the strongest Wassermann reaction. The next highest number of takes showed plus-minus Wassermann reactions, while the rest of the animals gave negative results. It is significant that animal S–b, which received six individual inoculations and showed three takes, had a doubtful Wassermann reaction, the one that received seven inoculations and developed four takes had a strong reaction. and the animal (P–9) that received eight inoculations but developed no take showed negative Wassermann reaction.

The fourth-month group is very small, yet it will be seen that one animal, which received seven inoculations and developed three takes, had a strong Wassermann reaction, while the other. which developed an ulcerative lesion, was negative. This places the ulcerative lesion in a group with the mild local lesion.

The factor of the form of yaws is emphasized in the next small group, the five-month group. The animal infected once in two places, that developed two takes but an intensive local exacerbation, showed a very strong Wassermann reaction; while another animal, which was inoculated seven times in seven places but had no take at all, gave a negative Wassermann reaction.

In the six-month group all factors involved except that of time are varied. The generalized yaws are shown to have given a strong Wassermann reaction, the ulcerative forms either negative or double plus, and the animals that developed local yaws, after six and seven individual inoculations with three takes, behaved similarly to those with generalized yaws. On

previous occasions the number of individual inoculations showed no influence on the results of the Wassermann reaction, provided they were all negative. In two cases (S–b and B–3), however, the negative takes occurred after a series of three positive takes, and the negative takes as quoted were the immune reactions described before. In such cases, when the organism reacts to the inoculation of *Treponema pertenue*, not by a typical yaw but only by an immune reaction, the body organism sensitized by local lesions that developed on previous inoculation may be stimulated to more intensive formation of the reagin.

In the next small group, the seven-month group, where the factor of time and the number of infections are constant and the number of takes does not vary much, the influence of the reaction that develops in a local yaw seems to be evident, in as much as three mild lesions did not produce a positive Wassermann reaction, while two typical yaws followed by three immune reactions produced in the animal a strong positive Wassermann reaction.

The eight-month group confirms the finding made in previous groups, that the number of individual inoculations is a minor factor.

In the nine-month group it can be seen that the generalized yaws and the local extensive yaws with regard to Wassermann reaction are similar, while of the local yaws one of the strongest reactions was found in an animal (A–2) that developed four takes as a consequence of six individual inoculations. The eleven-month group shows plainly that the number of successive infections has no effect and that the number of inoculations is of secondary consideration but the number of takes significant, in as much as the animal that developed four takes on eight inoculations at three successive infections gave a triple-plus Wassermann reaction; animal L–4, upon one infection and two takes following two inoculations, gave a double-plus Wassermann reaction; while the one (A–3) that on two infections and four inoculations developed two takes showed a single-plus Wassermann reaction.

The findings can be summarized as follows:

Of the five factors considered, the duration of the active lesion, the local reaction of yaw, and the number of the takes are primary factors in the strength of the Wassermann reaction in yaws. The number of successive infections showed no relation to the development or strength of the Wassermann

reaction, and the number of inoculations seems to be a secondary factor that enters only when the animal has previously had a yaws lesion.

The time factor enters, in as much as to a certain degree the Wassermann reaction becomes stronger with the increasing length of duration of active infection.

The form of yaws enters, in as much as the stronger the local reaction on the part of the affected tissues the stronger the Wassermann reaction; the milder the local reaction the weaker the Wassermann reaction.

The number of takes has a decided influence on the strength of the Wassermann reaction. The more takes the stronger the Wassermann reaction. The generalized yaws, the factor of repeated crops being eliminated, as evident from this table, comes under the heading of number of takes. That is, the repeated breaking out of yaws appears to count, in as much as the great number of individual skin lesions that take place in generalized yaws influence the Wassermann reaction in the same way as do numerous local takes.

The ulcerative forms, the so-called "tertiary," fall in with the mild local yaw.

As far as these conditions with regard to Wassermann reaction are known in humans, our results in experimental animals agree with the findings in human yaws; that is, the strongest Wassermann reaction is usually found in early generalized yaws, while the early local or the late so-called "tertiary" yaws give slightly positive or negative reaction. Three major and one minor factor are presented here which are responsible for the development and strength of the Wassermann reaction in yaws. Our understanding of the variations in the serologic findings in frambœsic patients in the same stage of the disease is hereby made easier.

Furthermore, the observation of the phenomenon of reappearance of positive Wassermann reaction following reinoculation in resistant persons(12) who, having been completely cured of yaws, fail to take, is hereby corroborated and amplified by experiments on animals.

THE INFLUENCE OF SUPERINFECTION UPON WASSERMANN REACTION IN EXPERIMENTAL YAWS

The effect of superinfection upon the Wassermann reaction in so far as it influences both the duration of the yaws process in experimental animals and the occurrence of various clinical

forms of yaws in monkeys, has been discussed in the preceding paragraphs. In Table 9 the effect of superinfection upon the Wassermann reaction is evident. The animals, having become negative without treatment with regard to the Wassermann reaction, again became positive within about a month after the first superinfection, and some of them earlier, while those that failed to take on the first inoculation and were reinoculated, having given persistently negative Wassermann, resulted in one strong and one weak Wassermann (monkey L–6 and M–5). The superinfection in these cases was performed during the period of reinoculability and was followed by the development of the typical local lesion. Therefore, it is not surprising that, upon the occurrence of a new lesion, what might be termed an artificially provoked recrudescence of the yaws process, the Wassermann reaction again became positive. In Table 9, however, the results of superinfection in animals that originally gave a positive Wassermann reaction were brought to a negative reaction repeatedly by treatment, and to a positive reaction by repeated superinfection following the treatment are given. The interesting finding is that the recrudescence and the relapses of the Wassermann reaction following superinfection happened during a period of resistance to superinfection; that is, the repeated superinoculations resulted in negative takes, and yet the Wassermann reaction became positive.

Another interesting finding is that during the process of repeated treatments and repeated reinoculations the response of the body organism of the experimental animal, both to treatment and to superinfection, became more and more sluggish with the increase in number of superinfections. As far as the behavior of the Wassermann reaction during the experimental infection is concerned, the following conclusions can be drawn:

1. The Wassermann reaction following an acute, uncomplicated, short-lived initial local yaw is irregular and inconstant, and occurs about the time when the local lesion becomes recognizable as a clinical yaw.

2. The Wassermann reaction is of short duration and for some reasons in certain cases does not become detectable.

3. Strong Wassermann reactions begin to make their appearance after the first infection has lasted or has been maintained for four months or more. The strength of the reaction depends on the number of experimental initial local yaws and on the intensity and extent of the local lesion.

TABLE 9.—*Showing the results of the Wassermann reaction in monkeys that were kept infected with yaws by repeated superinfection. The effect of specific treatment and of unsuccessful reinoculation in the resistant stage of yaws upon the Wassermann reaction is also shown.*

[—, complete hæmolysis; ±, trace of inhibition; +, slight inhibition; ++, about 50 per cent inhibition; +++, about 75 per cent inhibition; ++++, 100 per cent inhibition; 0, not done.]

Monkey No.	Date of first inoculation.	Date of examination, Wassermann reaction.	Result of Wassermann reaction.	Treatment.				Date of examination after treatment, Wassermann reaction.	Result of Wassermann reaction.	Date of reinoculation.	Clinical result of reinoculation.
				Commenced.	Finished.	Number of injections.	Total amount of neosalvarsan, in grams.				
A-2	III-4-25	II-16-26	+++	VI-17-26	VII-12-26	4	0.025	VII-17-26	—	VII-21-26	—
A-3	do.	do.	+	do.	do.	4	0.025	do.	—	do.	—
B-2	V-23-25	do.	++++	do.	do.	4	0.025	do.	—	do.	—
B-3	VIII-29-25	do.	++++	do.	do.	4	0.025	do.	—	do.	—
L-4	III-26-25	II-24-26	++	do.	do.	4	0.025	VII-20-26	—	do.	—
O-b	IX-12-25	II-25-26	—	do.	do.	4	0.025	do.	—	do.	—
D-9	VIII-29-25	II-18-26	++	do.	do.	4	0.025	do.	—	do.	—
H-16-b	VII-30-25	do.	+++	do.	do.	4	0.025	do.	—	do.	—
Positive controls			++++						++++		

Monkey No.	Date of Wassermann reaction.	Result of Wassermann reaction.	Treatment.				Date of examination after treatment, Wassermann reaction.	Result of Wassermann reaction.	Date of reinoculation.	Clinical result of reinoculation.	Date of Wassermann reaction.	Result of Wassermann reaction.
			Commenced.	Finished.	Number of injections.	Total amount of neosalvarsan, in grams.						
A–2	0	0	0	0	0	0	0	0	0	—	XI–26–26	0
A–3	IX–22–26	++	IX–27–26	X–26–26	6	0.03	XI–8–26	—	XI–8–26	—	do	
B–2	do	±	do	do	6	0.03	do	+	do	—	do	±
B–3	do	+++	do	do	6	0.03	do	—	do	—	do	+
L–4	VIII–26–26	±	do	do	6	0.03	do	—	do	—	do	—
O–b	XI–22–26	±	do	do	6	0.03	do	±	do	—	do	0
D–9	do	—	do	do	6	0.03	do	—	do	—	do	—
H–16–b	VIII–26–26	±	0	0	0	0	0	0	0	0	0	0
Positive controls		++++						++++				++++

4. Generalized yaws give a strong Wassermann reaction, such as the intensive or multiple local lesion.

5. Late ulcerative lesions (gangosa) give an indefinite, low-grade Wassermann reaction, similar to the finding in a feeble initial local yaw.

6. Upon spontaneous disappearance due to healing of the local yaw and following successful superinfection the Wassermann reaction again becomes positive.

7. Following specific treatment the Wassermann reaction becomes negative rather abruptly in a case of experimental yaws of short duration, and rather slowly in one of longer duration.

8. Unsuccessful superinfection with viable treponemas performed in the resistant stage of yaws has as a consequence a reappearance of the Wassermann reaction. When this process of alternating infection and treatment is continued for a long period of time, the promptness of disappearance of the Wassermann reaction after treatment and its reappearance after reinoculation are considerably decreased, and a stage is reached at times in which a low-grade Wassermann reaction persists practically unchanged by either of the two procedures mentioned.

PATHOLOGY OF EXPERIMENTAL YAWS IN PHILIPPINE MONKEYS

It is not intended to present in this section a thorough study of the pathology of experimental yaws. It was necessary, however, to study the histopathology of the lesions experimentally produced in the infected animals, as a confirmation of our observations and diagnosis of the clinical manifestations of yaws in experimental animals.

The description of the histopathology of the yaws lesions in man, as given in the literature by different authors, varies somewhat, apparently due to the difference in the age of the lesion examined. The most concise summary of the histopathology of frambœsia I found in the literature is that of Aldo Castellani and the late Albert J. Chalmers.(5)

The striking features in the histopathology of yaws are—

1. The cellular infiltration of the epidermis, which is responsible for the fundamental initial lesion of all early yaws manifestations; that is, the papule.

2. The cellular infiltration and œdema of the cutis, which is responsible for the elevation of the lesion above the surface of the surrounding skin and for the oozing of the lymph out of the lesions and the consequent formation of the crust.

3. The thickening of the epidermis and the downgrowth of the epithelial cells into the cutis, which is responsible for the papillomatous appearance of the early lesions.

4. The cornification and exfoliation of the superficial layers of the epidermis.

5. The excess or the lack of pigment.

While in the early lesions the first-mentioned three features are predominant, the later frambœsides mostly show the thickening of the epidermis, cornification, and exfoliation. The acanthosis is about the most constant phenomenon in practically all early or late yaws, and is found in lesions, on the margin at least, of the late, ulcerative deep type, where the granulation tissue and cellular infiltration are the most-striking features.

In as much as the pathology of early and late yaws lesions has been repeatedly studied by various investigators, we shall not go into the details of the description of these lesions. Some glimpses into the histopathology of experimental yaws are afforded in the attached plates and in their respective explanations. It is, however, not out of place to mention the pathology of experimental gangosa, as it is believed that this phase has not been studied very exhaustively. The contribution is not an exhaustive report on the histopathology of gangosa, but several new points can be presented; they are of importance in the interpretation of the pathogeny of this peculiar condition. It has been noticed at the clinical examinations made during the development and course of experimental gangosa that the process affects a very extensive area of the mucous membrane, far greater than the area which actually undergoes the real ulceration in any stage of the development and course of the condition. This clinical observation on experimental gangosa has been confirmed at autopsy on gangotic animals performed with a view of obtaining some information with regard to the pathogeny of gangosa. It was further confirmed by histopathologic findings. These findings will explain the considerable variation in topography of gangotic ulceration in human patients who present themselves at a stage of full development or at a more or less advanced stage of this condition. The nose, the soft palate, and the larynx have been clinically observed to exhibit an ulcerative process and, because of the location of this ulceration, these various parts of the upper respiratory system were claimed to be the original seat of the initial gangotic lesion. My observations on experimental gangosa led me

to believe that there are only two ways in which the gangotic process begins and, consequently, two forms of this condition can be observed in experimental animals.

In view of these observations on animals the conclusion can be drawn that gangosa in human patients, in whom the gradual development could probably never have been followed from the very beginning to the well-developed stage, should be divided into two forms, the pathogenesis of which is similar to that in animals. That the early yaws lesion enters the nostril and the nasal mucous membrane has been observed repeatedly, as mentioned above. It has also been observed that the skin lesion, either spontaneously or due to therapy, disappears first while the nasal lesion persists. Histopathologic study explains the mode of spreading of early yaw lesions on the mucous membrane, not only of the mucocutaneous border (that is, onto stratified epithelium), but also on the mucous membrane covered with high cubic or cylindric epithelium. The early yaws lesion propagates itself in a similar manner on the mucous membrane as it does on the skin; that is, in the most superficial layer of the mucous membrane (in the epithelium) which gradually undergoes necrosis and, consequently, the mucous membrane becomes inflamed, showing œdema and cellular infiltration (Plate 29, fig. 2). Not all of the area of the inner lining of the nasal cavity affected necessarily undergoes ulceration, and it is only in certain places that offer favorable anatomic conditions that the ulceration ultimately develops. From our experiments it is clearly evident that the process may have trespassed, with or without partial healing, a considerable area of the inner lining of the nose before the allergic state of the entire body, which is responsible for the ulceration, was reached. It can be easily understood, therefore, that the frambœsic process may reach the soft palate and even the larynx before the allergic condition sets in, and then the location of the most intensive ulceration is by no means necessarily the initial lesion of gangosa. The ulceration, therefore, may take place in sections of the upper respiratory system, between the nostrils throughout the nose, pharynx, and larynx. The finished product of this process, which is the ulceration, may be considered clinically far more important than the initial lesion, and the fact that patients usually do not present themselves at the clinic until this ulceration has been accomplished is responsible for the faulty interpretation of pathogeny of gangosa as given in the past literature. It is no more necessary that the most-

advanced lesion in gangosa is the primary lesion than it is necessary to consider great metastases of cancer in the liver as an initial lesion and to disregard the comparatively smaller and at first sight less-significant cancerous ulcer of the pylorus. The second form of gangosa is the late ulcerative skin lesion located in the neighborhood of the nose, which spreads by continuity through the roof of the nose (Plate 17, fig. 1).

STUDY OF IMMUNITY TO YAWS, BASED ON EXPERIMENTAL EVIDENCE IN PHILIPPINE MONKEYS

INTRODUCTION

One might judge the existence of immunity in animals infected with yaws, first, by percentage of positive takes upon superinoculation; second, by duration of the incubation; third, by duration of the lesion; fourth, by the character of the lesion that develops on superinoculation or reinoculation. From the records of normal monkeys inoculated with yaws material it is evident that the percentage of takes, unless inoculation be repeated, may be misleading in judging the immunity, in as much as 100 per cent of takes would not always result upon one attempt at inoculation. There are, furthermore, certain variations in the incubation period and great differences in the duration of the lesion in the individual animals. This fact cannot be explained as due to differences in the inoculum, because the same monkey inoculated in symmetric places of the body with the same amount of the same inoculum showed considerable variations, both in the incubation and in the duration of the corresponding lesions. As a general rule and on the average, the florid stage of the lesions in soft parts of the skin, such as the scrotum, lasted longer than that induced by artificial inoculations in other parts of the body. However, we have repeatedly seen, in monkeys inoculated for the first time, as mentioned before, that the lesion stopped and regressed in any of the stages of development of the initial yaw. It follows necessarily that, no matter how much they may be influenced by immunity or relative resistance, neither the duration of the incubation period and of the lesion nor the rapid regression of the lesion alone can be safely taken as a criterion for judging the immunity to yaws of monkeys; they are uncontrollable factors, from the experimental point of view. The type of the lesion, however, if its character be taken into consideration, appears to be significant and is as reliable as can be expected.

INTERPRETATION OF RESULTS OF SUPERINFECTION IN YAWS ANIMALS AS A TEST FOR IMMUNITY

In order to prevent the interference of errors in inoculation due to technical or other reasons, the following precautions were adopted.

Superinoculation and reinoculation of yaws animals were performed as described in the beginning of this paper; that is, by intradermal injection. In a very few instances the first attempt at inoculation resulted negative while a subsequent inoculation gave positive results. This happened a few times in the early period of our experimentation, but not in the latter part of the period over which these experiments extended. It is quite possible that the few early failures were due to imperfect technic, but there is an indication of definite adaptation to the monkey's organism of the strain experimented with, in as much as, after the strain had been passed through monkeys for sixteen months, spontaneous generalization after a single inoculation occurred. At the time when the bulk of the superinfection experiments were performed, no failures of takes were noticed. Nevertheless, to assure myself that the material used for superinoculation contained viable and infective treponemas, at least one control (more frequently two) was inoculated simultaneously and with the same material with which a given group of yaws monkeys had been superinfected. Besides this precaution, and in spite of the fact that control normal animals developed the typical yaw, animals that were superinfected and in which the superinfection failed to take were again subjected to superinoculation after a period of one month or more, which time was considered sufficient for the development of the lesion, in view of the fact that we had previously found in a series of monkeys that the incubation period rarely exceeded four weeks. Aside from this double precaution, a safe criterion was used for recording the results of the superinoculation as negative. It was noticed that immune animals superinfected in the early part of the resistant stage invariably presented what is referred to in this paper as specific swelling. As mentioned above, in the description of the development of the initial yaw, the œdematous swelling that persisted for twenty-four hours following the intradermal injection of not more than 0.5 cubic centimeter of inoculum disappeared within a day and there was a lull at the place of inoculation for about twenty-six days, when the lesion started to develop. The specific swelling which may be referred to as immune reaction, due to its resemblance

to the positive luetin reaction in syphilis, developed in immune animals rather early; that is, within a few days after injection and apart from the initial traumatic œdema. Size and symptoms increased rapidly and disappeared quite as rapidly. This reaction as suggested by the term here used, "specific swelling," consisted of noninflammatory œdema, so that it could not be interpreted as a reaction due to the bacteria admixed with the inoculum. Sometimes slight necrosis was noticed. Furthermore, the normal control animals inoculated simultaneously and with the same inoculum did not exhibit such reaction, a fact which further corroborates my contention that this reaction was due to *Treponema pertenue* and not to bacteria present in the inoculum. With this in mind all of the tables must be interpreted.

The appearance of late ulcerative frambœsic lesions shows a condition of body organism having a tendency to localize the yaws process and to keep it from further spreading. That this is due to the state of the superinfected organism and not to the change in the strain of the treponemas is shown by the fact that these lesions developed only in infected animals, while fresh, normal control animals, inoculated at the same time with the same inoculum, developed the typical initial yaw. We have therefore two manifestations of the allergic state following repeated successful inoculation with yaws material. The consequence of the first allergic state is an extensive lesion resembling the initial yaw but far more extensive and intensive, containing treponemata in enormous numbers and followed by generalized yaws; the second allergic state is responsible for the development of ulcerative lesions such as one meets with in late, or so-called "tertiary," yaws in man. The last-mentioned condition indicates an immunity which, while not strong enough completely to suppress the development of the lesion, localizes it, and the tissues react by excessive granulation; at the same time the treponemas are kept at bay.

From the findings in superinfected animals one can reconstruct the pathogenesis and immunity of yaws in man. The description of the incubation period, the course of the initial yaw, the generalized yaws, the frambœsides, and the late forms in monkeys leaves no doubt that, under some circumstances, the disease runs the same course in monkeys as it does in man. The primary yaw develops and runs the course as described above. Superinfection in spontaneous yaws of man from within, in experimental animals from without, under favorable aller-

gic conditions of body organism, produces a generalization, and crops of yaws appear. We have noticed that in superinfected monkeys extensive, local yaws developed at times, as a consequence of superinfection, far more extensively and intensively than did the first yaws lesion. This seems to indicate clearly that a state of greater susceptibility was the consequence of the previous inoculation at the time of the following superinfection. Indeed, as mentioned before, this allergic state was suggested by the fact that local exacerbations and local metastases occurred without superinfection, in one monkey inoculated for the first time, that were of greater intensity than the original lesion (Plate 5, fig. 1).

In other monkeys the result of repeated superinfection was a development of lesions identical with those encountered in humans in the late stage of yaws; that is, deep, ulcerative, granulating, slowly developing (not healing) lesions. This again indicates an allergic state of the entire body in the late period of yaws, because the mutilating lesion developed not only at the place of superinoculation, but also in places remote from the point of superinoculation, as an exacerbation of the vanishing original frambœsic process.

One cannot avoid the impression that in generalized yaws the increasing immunity is responsible for the gradual restriction of the successive early metastatic lesions to more and more superficial layers of the skin; that is, for the gradual change from crops of typical yaws to crops of superficial, evanescent, early frambœsides. This impression is supported by the fact that yaws monkeys cannot be superinfected when the cropping out of yaws has ceased, although the general manifestations of yaws present at the time of superinoculation may last a considerable time after the unsuccessful superinoculation.

From the observations made on the development of the resistance to superinoculation and reinoculation as well as on the healing of the lesions in monkeys, one can draw certain conclusions with regard to the mechanism of the immunity to yaws.

Even though it may be possible to produce immune sera by artificial immunization of laboratory animals with *Treponema pertenue,* there is no evidence of humoral immunity to yaws in man or in monkeys.

Against humoral immunity being responsible for the resistance to superinfection speaks, first of all, the common observation that monkeys become resistant to superinoculation long before the already existing early lesions heal. These lesions

are not secluded from the general system of circulation of the blood and the lymph, as the profuse oozing and the bleeding of their surfaces clearly indicate.

In not less than six instances we failed to observe any effect whatsoever upon the yaws lesions of animals during the susceptible stage of a direct injection into the lesions of blood serum collected from resistant monkeys that had gone through a generalized yaws infection. This confirms the observation of Sellards and Goodpasture(29) made on human yaws.

When superinfections are continued on the same animals, sooner or later a stage is reached in which the inoculated treponemas, although proved to be virulent and viable in normal control animals, fail to produce lesions recognizable as yaws. The state of nonreactivity on the part of the body organism toward incorporation of *Treponema pertenue* was more nearly complete and was reached much more suddenly in those animals that went through generalized early yaws than was the case with the animals that upon superinfection developed deep ulcerative lesions or a local yaw only.

The development of complete and protective immunity is very slow, however, and the fact that frambœsides or late lesions develop is a sign of partial immunity, in the broad sense of the word. Consequently, the difference in susceptibility to generalized or late ulcerative yaws of the human organism and that of monkeys lies in the quickness and promptness with which the human body organism responds to the infection by one or the other of the two allergic conditions. The monkey's body organism is more sluggish in this respect, but can be stimulated by artificial superinfection.

With regard to the results of superinfection three types of conditions were observed, namely:

Type A.—Animals inoculated for the first time developed typical, initial, local yaw which ran its course without exacerbations and healed in a relatively short time. The second and even the following inoculations of the same animals resulted in the development of a lesion identical with that upon first inoculation or with that in inoculated normal control animals. Continued superinfections produced local yaws lesions of mild degree and, ultimately, only a specific swelling which rapidly disappeared, or no lesion at all. Control animals inoculated at the same time with the same inoculum gave typical takes.

Type B.—Animals inoculated for the first time developed typical initial local yaw, which ran its course without exacer-

bation. About the time when this lesion was vanishing and on the verge of healing the superinoculation was made. The second inoculation resulted in a lesion identical in character with lesions obtained in normal control animals, but was far more extensive and intensive. In the case of spontaneous generalization of local experimental yaw, an extensive local exacerbation took the place of superinoculation. A general eruption of typical metastatic yaws of early evanescent frambœsides and late persistent frambœsides followed in these animals. When crops of general yaws lesions no longer occurred, repeated inoculations were made which resulted in a specific swelling at the point of injection, topped with a noncharacteristic scab, or no lesion at all. No treponemas were found in the last-mentioned lesions. Normal control animals inoculated at the same time with the same material developed typical initial local yaw.

Type C.—Animals in which inoculation resulted in local lesions of long standing with occasional mild local exacerbation, and in which the yaws process was further kept alive by repeated superinoculation, did not develop generalized metastatic eruptions of typical early yaws or early frambœsides, but developed in due time late persistent frambœsides (keratoderma plantare), gave repeatedly positive though not typical takes, and sooner or later the inoculation resulted in a deep ulcerative lesion, slowly spreading, not healing while active, and when healing took place it was from the periphery of the lesion. Very few treponemas were found in these lesions. Some of these animals developed gangosa as a result of such lesion in the vicinity of the nose, while others developed gangosa from a residual lesion inside of the nose upon reinoculation on the eyebrows. These animals while the ulcerative process was still progressing took repeatedly, but when the process was arrested the superinoculation resulted in a feeble lesion or specific swelling of short duration, or no lesion at all, in spite of the fact that control animals inoculated at the same time with the same material developed a typical initial yaw.

From Table 10, showing the gradual development of immunity as demonstrated by superinfection, it can be seen that yaws monkeys in which the local frambœsic process lasted five months or less took invariably, showing a typical initial yaw upon superinfection. In animals in which the local process remained alive seven months or more the results of superinoculation were invariably negative; that is, a specific swelling developed which disappeared rapidly, or no lesion at all.

Comparing the results of repeated superinfections (Table 10) with the results of one superinfection on one hand and with the results of superinoculation in generalized yaws on the other, it can be seen that the repeated superinfection has no apparent effect on the early development of immunity. The generalization of yaws (Table 11), however, distinctly hastens the development of resistance to superinoculation. In the animals, however, that developed late ulcerative lesions the development of complete immunity was greatly delayed (Table 12).

The fact that immunity in experimental yaws begins to be noticeable in the sixth month after inoculation and the fifth

TABLE 10.—*Showing the development of immunity in yaws-infected Philippine monkeys.*

[—, negative take; +, positive take; ±, feeble take.]

Monkey No.	Months after original inoculation.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
L-6	+													
M-5	+													
T-4	+													
J-13	+													
A-5		+												
D-10		+												
G-7		+		+	±	—								
F-13		+		+										
E-13		+				+								
K-3		+												
L-5		+												
K-2			+		+		—		—					
B-1			+			+				—		—		
B-2			+			+				—		—		
B-3			+				—		—					
H-17				+		—								
J-11				+		—								
F-12				+		—								
C-2				+		—								
P-10				+				—	—	—				
M				+										
S-b				+			—							
O-b						—		—						
F-11						—								
M-3						—		—						
M-2						—								
A-3							—					—		
D-9							—		—					
A-2								—		—		—		—
H-16-b								—						
D-8											—		—	
L-4												—		

TABLE 11.—*Showing the development of immunity in monkeys with generalized yaws.*[a]

[+, positive take after superinfection: —, negative take after superinfection; exac, exacerbation; gen, generalization; reg. met, regional metastases.]

Monkey No.	Result of first inoculation.	Months after original inoculation.																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
P–8	Initial local yaw				+	gen			—												
N–8	do		exac	reg. met	gen		—			—											
T–1	do	+		exac	—, gen			—													
G–6	do		+—, exac	—, gen	—																
C–3	do				+	exac gen	—														
E–15	do	+			exac	gen			—												

[a] The symbols are placed in the months in which the superinfection, the exacerbations, or the generalization took place.

TABLE 12.—*Showing the development of immunity in monkeys with late ulcerative lesions.*

[+, positive take; ± feeble take; —, negative take.]

Monkey No.	Result of first inoculation.	Months after original inoculation.																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
C–1	Initial local yaw	+														+, ulcer				±	
J–10	do				+, ulcer						±										
H–18	do		+		+, ulcer	—															

month after development of the initial yaw is further brought out by the observation that generalization of the yaws process, either spontaneous or induced by superinfection, has invariably occurred from the third to the fifth month, after initial inoculation, never later. Thus the findings made on superinoculations are supported by the observations made on those animals in which general manifestation of yaws took place.

In connection with the late ulcerative type of lesion the findings are significant. Once the ulcerative lesion has developed and persisted for some time, the following superinfection still results in a lesion, recognizable as a yaw, in which treponemas can be found. We have designated this lesion as feeble, for the reason that its appearance was not that of typical initial yaw, although oozing and crust forming occurred. It always was a short-lived lesion that did not spread so rapidly and extensively as is usual with the initial local yaw, and was characteristic because of the extreme swelling surrounding the lesion. On the other hand, it did not show the character of an ulcerative lesion. This seems to indicate that after the ulcerative lesion had persisted for some time immunity has developed to a higher degree and an ulcerative lesion did not result so completely typical as the one already existing. In other words, the period during which ulcerative forms of yaws spring up is limited, and is shorter than the duration of the existing ulcerative lesions.

The protective immunity in any persistent form of yaws toward new superinfection rises more quickly and to a higher degree than the body's power with regard to the healing of the existing lesions and, to a certain degree, independently of it. It was observed that in the case of an ulcerative lesion the feeble lesion that developed as the result of subsequent superinfection showed far more rapid healing than did the existing ulcerative lesion itself. Metastatic lesions of generalized yaws may persist and harbor viable treponemas months after the animal becomes completely resistant to superinfection. On the other hand, an initial local yaw heals at a time when the animal is still fully susceptible to superinfection.

These findings are significant, in as much as they show that the difference with regard to immunity between the local yaw and generalized early yaws is quantitative. If the local initial yaw persists for a long time, or if repeated development of successive initial yaws is experimentally induced, immunity after a long period of time develops as complete as is possible

in frambœsia. If the initial yaw is followed either by local yaw due to superinfection which develops into an extensive and intensive local lesion or by extensive spontaneous local exacerbation, the natural resistance of the body of the animal to generalization of yaws is lowered. Within a month or so after this allergic phenomenon the first crop of generalized yaws occurs. Without further superinfection this first crop of typical yaws may be followed by a second, a third, and even a fourth crop of specific efflorescences; but in the last crops superficial frambœsides are usually admixed with the eruption of typical yaws, and the ultimate crop, or even the penultimate one, may be composed exclusively of superficial evanescent frambœsides. By this time the immunity had developed to such a degree that upon superinfection neither typical or mild yaw nor late ulcerative lesions developed. In other words, animals that have gone through repeated crops of generalized early yaws manifestations did not develop late ulcerative, so-called "tertiary" lesions, but were at the end of the generalized dissemination immediately as completely immune as is possible in frambœsia. The superinoculation, which was well controlled and checked up by simultaneous inoculation of normal animals, resulted in nonspecific œdematous swelling, possibly with a slight superficial necrosis, or no lesion at all. The resistance to superinoculation set in as soon as the metastatic yaws ceased to crop out, but long before the last remnants of generalized manifestation had healed, and a considerable time before treponemas had disappeared from these lesions.

In order to place the findings recorded in our study of immunity to yaws in a system two kinds of protective immunity in yaws, apart from healing, must be assumed; one is the lytic and the other is the neutralizing immunity. The first type of resistance in monkeys (type A) shows slow development and both immunities (that is, the lytic and the neutralizing) develop and run together as a single line. In other cases (type C) the two kinds of immunity do not run parallel, but the lytic immunity develops to a higher degree than does the neutralizing one. Consequently, deep ulcerative lesions developed, because the majority of the treponemas have been dissolved and their toxic substances liberated, but the body tissues are not able to neutralize these toxic substances and react, either locally with excessive granulation or in remote places by excessive hyper-

keratosis. This theoretical system forces itself on one's mind because of the great similarity of the immunity in yaws to that in vaccine virus.

The healing of a yaws lesion is staged locally within the lesion itself, and in the surrounding tissues in such a manner that the deeper layers of the affected skin heal first and the healing process progresses toward the surface of the skin. In consequence of this the transformation of the typical yaw into the fourth, fifth, and sixth stages of the local yaw is accomplished, and lesions result that may be designated as local or regional frambœsides.

If the throwing off into the blood stream of the treponemas from the mother yaw takes place at a time when the resistance of the skin tissues has developed to a certain degree but not to the full intensity, the treponemas localize in the epidermal layer of the skin, as is their nature, but do not multiply as extensively as they do in a fully susceptible skin. Consequent upon the resistance of the skin tissue they are barred from the deeper layers of the cutis and the lesion remains restricted to the superficial parts of the skin. The result is the appearance of metastatic, otherwise generalized frambœsides.

The observation made in the course of our experimentation that inflammation and pus formation at the place of inoculation prevent or delay the development of yaws lesions points to the important part which leucocytes play in the destruction of treponemas. An extensive disintegration of the leucocytes was noted by Goodpasture(8) to take place in yaws lesions healing as a consequence of neosalvarsan theraphy. It can be easily suspected that this phenomenon helps in the destruction of the treponemas in the lesion.

Everything points to the conclusion that not humoral but cellular immunity is responsible both for the resistance to superinfection and for the healing of the existing lesions, and that the leucocytes play an important part in the healing of the lesions. The healing is independent of the resistance to superinfection or reinfection, or else a local yaw could not heal in the stage of infection when the animal is susceptible to superinfection, even at the very same place in the skin where the yaw has healed. Likewise, the fully developed resistance to superinfection has no immediate effect upon the healing of the existing

early yaws lesion or on the disappearance of the treponemas present therein.

CONCLUSIONS

The second of the three problems set forth in the introduction finds its solution in the following summary.

Immunity to yaws in Philippine monkeys exists. It presents itself in the following ways: First, as resistance to superinoculation; second, as resistance to generalization of the yaws process; third, as a modification of the yaws lesions, both local and generalized.

The resistance to superinoculation is slow in development in a case of persistent local yaw.

The resistance to superinoculation develops much more rapidly as a consequence of generalized manifestations of yaws than it does following local yaws.

The generalization, either spontaneous or due to superinoculation, takes place upon superinfection performed not later than five months after the original inoculation and not sooner than three months.

The resistance to superinoculation is not noticeable until from six to seven months after the original inoculation in case of the local yaw, and immediately upon termination of the cropping out of the generalized yaws in cases of metastatic yaws.

In case of ulcerative lesions the resistance to superinoculation is much delayed.

The resistance to superinoculation lasts at least three years.

As a phenomenon of immunity, in the broad sense of the word, two allergic states exist; namely, one which has the character of a negative phase or a higher susceptibility to superinoculation or susceptibility to excessive local exacerbations. This condition may be followed by general clinical manifestations of yaws; the second allergic state is of the character of an anaphylactic condition in which the body tissues react to superinoculation by excessive granulation and ulceration at the place of inoculation or at the place of the residual local yaw, and by hypertrophic or atrophic skin lesions in places remote from the point of inoculation.

The resistance to superinoculation is developed at the time when yaws manifestations still persist in the form of local

yaw, metastatic yaw, frambœsides, or ulcerative lesions, and when they still contain viable treponemas.

Complete healing of local yaws lesions does not necessarily mean the onset of resistance to superinfection, or vice versa.

Evanescent early and later persistent frambœsides, as well as ulcerative lesions, are signs of partial immunity.

REINFECTION: IMMUNITY AFTER TREATMENT

Having established the fundamental points concerning immunity as it developed in Philippine monkeys in consequence of the various clinical forms of yaws manifestations, we proceeded to the solution of the third problem, as indicated in the introduction; that is, to the question whether the resistance of the yaws-infected monkeys lasts only during the stage of infection or whether it persists after a thorough specific treatment.

(*a*). *The influence upon immunity to yaws of treatment given in the resistant stage of yaws infection in Philippine monkeys.*—The arrangement of the experiments that fall in this section of investigation was as follows: Animals that developed local yaw, which either persisted spontaneously or was kept alive by successive superinoculations until such time as the animals would no longer take but developed only specific swelling of various degrees or no lesion at all as a consequence of superinoculations, were given intramuscular injections of neosalvarsan. After the treatment with neosalvarsan the animals were reinoculated with yaws.

A similar procedure was adopted in the case of monkeys that had gone through generalized yaws and upon superinoculation proved to be resistant.

(*b*). *The influence upon immunity of treatment given during the susceptible stage of yaws in Philippine monkeys.*—In another series of experiments the effect of the specific treatment upon reinoculability was tested when given during the early stage of the infection; that is, during the susceptible stage. Treatment was given in these cases at intervals varying from two to seven months after the original successful inoculation. Reinoculation was performed not less than seven months after the initial inoculation; that is, a period of time was allowed to elapse after the initial inoculation, which in animals with

infection uninterrupted by treatment was found sufficiently long to bring about resistance to superinoculation. The treatment was given intramuscularly at the intervals indicated in the protocols. Complete sterilization by "dosis sterilisans magna" was not attempted in these experiments, but the total dosis administered by repeated injections reached or exceeded the amount of 0.01 gram of neosalvarsan per kilogram of body weight. This arrangement of the treatment was adopted owing to the high toxicity of arsenic to our experimental animals. The total amount of the drug given to our experimental animals, therefore, exceeded slightly the amounts known by clinical experience to be sufficient to bring about complete cure of early cases of human yaws.

As the result of the treatment whatever remnants of specific lesions may have been present in these animals from previous inoculation completely disappeared after one or two injections of neosalvarsan. This was true, not only of the specific frambœsic lesions, but also of the nonspecific manifestations, such as alopecia. It was noticed that following the treatment (as a matter of fact, before the entire treatment was accomplished) the hair in places where alopecia had set in began to grow and the animals gradually assumed a normal appearance. They seemed stronger and livelier than before treatment. The serological examinations that gave positive results before treatment were repeated and found negative. It is believed that all the precautions within man's power were taken, and the animals were considered cured. This assumption was furthermore strengthened by the experience accumulated during these investigations that monkeys are less susceptible to generalization of yaws than are humans; and that the frambœsic process left untreated, either local or generalized, lasts in the monkeys a far shorter time than in man and heals spontaneously much more quickly. It was concluded, therefore, that frambœsia will be even more amenable to the specific treatment in monkeys than in man; particularly in view of the observations made with regard to healing of late ulcerative lesions and late persistent frambœsides, which lesions healed in a comparatively short time following a single injection of neosalvarsan. It is a well-known fact that late frambœsic lesions in man do not respond to specific treatment with such great rapidity as the early lesions do.

TABLE 13.—*Showing the results of reinoculation of Philippine monkeys after treatment given during the resistant stage of yaws.*[a]

[Tr, treatment; —, negative take.]

Monkey No.	Months after original inoculation.																	
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
M	tr	—																
E-15[b]	tr	—																
G-6[b]	tr			—														
F-13	tr				—													
C-2		tr				—												
O-b		—	tr	—														
J-11			tr				—											
F-12			tr				—											
H-17			tr				—											
B-3	—		—	tr	—		tr		—				—					
D-9	—		—	tr	—	tr		—										
H-16-b		—		—	tr	—												
B-2				—		—	tr	—		tr		—				—		
A-3	—					—			tr	—		tr		—				
L-4						—		—	tr	—		tr		—				
A-2		—		—		—		—	tr	—								
D-8					—		—		tr									—

[a] Results of inoculations performed on these animals previous to the seventh month can be found in schematic protocols.

[b] Generalized yaws.

TABLE 14.—*Showing the results of reinoculation of yaws monkeys, treated in the susceptible stage of infection.*

[Tr, treatment; +, positive take; —, negative take.]

Designation of monkey.	Period of treatment and reinoculation, in months.						
	1	2	3	4	5	6	7
J-16	tr						+
E-16		tr					+
E-17		tr					+
T-10			tr				—
M-4			tr				—
T-4				tr			—
B-4				tr			—
J-13					tr		—
O-c					tr		—
A-5					tr		—
M-5					tr		—
L-6					tr		—
L-5					tr		—

From Tables 13 and 14 the following conclusions can be drawn:

When treatment was commenced during the first or the second month (after the original inoculation) the animal remained

susceptible to reinoculation and developed local yaw when inoculated seven months after the original inoculation. The rest of the animals that received treatment later than the third month could not be reinoculated in the seventh month after the original inoculation.

Naturally, as already mentioned, the infection was not terminated by the first injection of neosalvarsan and the immunization process evidently continued for some time after the initial therapeutic injection. It took one week or more before the lesions completely disappeared.

Summarizing the events of experimental infection, superinfection, and reinfection in monkeys as far as concerns this study of immunity, we see that the original inoculation is followed by a long incubation, during which time no signs of local reaction or of serologic changes are noticeable. Following the incubation a lesion begins to develop in the form of a papule located in the most superficial layer of the skin (Plate 28, fig. 1) and containing so few treponemas that, as experience taught us, scraping the lesion at this period in a search for treponemas revealed these, but the progress of the lesion was thereby stopped and the infection terminated. In the early stage of development the yaw remains superficial, the treponemas multiply slowly, and the Wassermann reaction is indefinite or slightly positive. Specific treatment given at this stage will leave the animal reinoculable.

From the third to the fifth month the infection gains the upper hand. The yaws process has reached the deeper layers of the skin (Plate 28, fig. 2) and the treponemas have multiplied considerably. The Wassermann reaction becomes stronger the nearer the infection approaches the fifth month. Local exacerbations take place during this period, assume at times an unusual extent and intensity (Plate 3), and contain enormous numbers of treponemas. Generalization of the yaws process takes place between the third and the fifth month. Then gradually, even though rather suddenly, the full resistance to superinfection appears. It is heralded sometimes by a feeble lesion upon superinfection in case of local yaw, and by appearance of frambœsides in case of generalized yaws.

If our supposition that the late penetration of the treponemas into the deep tissues of the skin is responsible for the delay in the development of immunity is true, then it must be possible not only that susceptible animals can be immunized by sub-

cutaneous infection without development of yaws, but also that the immunity conveyed in this manner should develop much more quickly than it does following development of a local yaw and about as rapidly as does that following a generalized yaws eruption. The experiment discussed in the following paragraph proves this contention. (See Table 15.)

TABLE 15.—*Showing results of subcutaneous immunization without yaws skin manifestation.*

[+, positive take; —, negative take; +++, strong Wassermann reaction; ++++, very strong Wassermann reaction; 0, not done.]

Monkey No.	Subcutaneous injection of yaws material.			
	First.	Second.	Third.	Fourth.
D–13	II–3–27	1 week	2 weeks	5 weeks
D–12	do	do	do	do
N–12	do	do	do	do
Normal control	0	0	0	0
Do	0	0	0	0

Monkey No.	Intradermal infection with yaws material.			Wassermann reaction.
	Time after—		Result.	
	First injection.	Last injection.		
D–13	6 weeks	1 week	+	0
D–12	16 weeks	12 weeks	—	+++
N–12	do	do	—	++++
Normal control	VI–4–27	0	+	0
Do	III–15–27	0	+	0

Three monkeys received, within five weeks, four subcutaneous injections of yaws material containing numerous treponemas. No lesions developed at the place of the subcutaneous injection, but a local swelling did appear. Following this process of immunization one of the three animals was infected intradermally with yaws one week after the last injection and six weeks after the first subcutaneous injection. In due time this monkey developed a typical yaw, showing that the animal was still susceptible at the time of inoculation. The other two animals were infected in a similar way four months after the first sub-

cutaneous injection and three months after the last subcutaneous injection. They failed to develop a local yaw or any other signs of yaws infection, even though the same material when used for intradermal infection of a normal control animal brought about the development of typical yaws.

Direct conclusions can be drawn from this experiment; that is, that immunity develops as a result of subcutaneous inoculation without clinical development of yaws, and that the time of development of immunity is shortened. It develops later than six weeks and earlier than four months after the initial subcutaneous injection of yaws material. It is interesting to note that the animals gave a rather strong Wassermann reaction without ever showing any sign of clinical yaws at all.

Evidence seems to be afforded in this experiment that our contention expressed previously is correct; that is, that during the first two months or so no immunity develops as a consequence of intradermal inoculation and that the immunity begins to develop at the end of the second month and in the third, fourth, and fifth months, when the yaws have reached the deeper layers of the skin. By the arrangement adopted in this experiment we turned aside the period of incubation and the period during which the yaws remain superficial and placed the experimental animals right in the third or fourth month of the yaws process with regard to immunity. Once the fact has been established that immunization to yaws can be carried out by subcutaneous inoculation without production of a yaw, which process may be called a paradermal immunization, there evolve further problems with which, however, we are not concerned at present.

One is naturally tempted to draw far-reaching conclusions, and further interesting investigations are suggested. This finding gives food for thought. The question may be asked: Is it advisable in treponematous infections to terminate the infection in the early stage by abortive treatment, or is it preferable and in the interest of the individual affected to allow the body to reach that stage of infection which still is curable but leaves the individual in a condition of resistance to further infection and to late manifestations?

Is it possible to crowd within the short time of the incubation of general dissemination of yaws a sufficient number of subcutaneous injections without the production of the clinical lesion that would prevent the eruption of general clinical manifestations due to the immunization process itself? In other words,

will it ever be possible to work out a method of immunization against treponematous infections similar to that in use for immunization against rabies, during the incubation period?

These are, of course, suggestions that necessarily occur, and much experimentation will be necessary before any answer can be given. Fortunately, we possess in the monkey an excellent experimental animal, so there should be no great difficulty in working out this problem on animals.

LATENT INFECTION AND FRAMBŒSIC LYMPHADENITIS [*]

By latent infection we mean that stage of treponematous infection when the clinical manifestations and other symptoms have disappeared, either spontaneously or as the result of treatment, but the treponemas have survived within the tissues of the body and again produced later, after a period of latency, clinical manifestations and symptoms. It is in this sense that the term latent infection is to be interpreted in this section of our experimental work.

In order to ascertain whether or not such condition as defined above exists in experimental yaws we proceeded in two directions:

First, we watched for a relapse in those monkeys that had either been cured or been healed spontaneously. This observation was continued for a period of months.

Second, we endeavored to ascertain the survival of treponemas in those organs of experimental animals that, in a disease like frambœsia tropica, would naturally be the storage place of treponemas.

The second investigation was carried on in two ways; namely, (1) we searched for treponemas by means of microscopic dark-field examination; and (2) we endeavored to prove their presence and at the same time their viability by means of inoculation into healthy experimental animals.

As a focus of treponemas where they would most likely be found we took the regional glands corresponding to the active, healing, or healed lesion. It has been known since the time of Castellani's early investigations on yaws that *Treponema pertenue* may be found in the glands corresponding to the frambœsic lesion in experimental animals.

[*] C. M. Hasselmann, formerly of the University-Clinic for Dermatology, Syphilis, and Genito-urinary Diseases, Frankfurt a. M., Germany, and of the Tropical Institute at Hamburg, collaborated in the preparation of this section, pages 297 to 305.

This fundamental fact having been established it remained to be seen what importance it bears upon the maintenance of latent infection and subsequent relapses. It is not to be wondered that a microörganism which penetrates as easily as does *Treponema* will find its way through the lymphatic spaces into the corresponding regional gland. There may be merely a mechanical deposition of the treponemas in the regional gland where they remain for some time and are destroyed then and there, or it may mean that they are deposited there to stay, or they may pass through this gland and work their way through to other parts of the body. It becomes important, therefore, for the interpretation of the positive findings of treponemas in the regional glands, whether they remain in the gland for a sufficient length of time and permanently establish themselves in the lymphatic apparatus or not. Furthermore, the question arises whether or not, if they establish themselves in the lymphatic apparatus, they are capable of invading the body from this hiding place and later causing relapses. Our investigations in this direction are sufficiently extensive to allow of some conclusion.

The result of our observations of the spreading of the early primary yaw leaves no doubt that the treponemas travel from the original place of inoculation through the lymphatic spaces and radiate toward the periphery in a fanlike manner and progress (equal conditions of the skin over which they travel being granted) at about the same rate, thus causing the yaws healing in the center to spread in a more or less regular circle toward the top of the head, when inoculation was performed on the eyebrow.

They spread toward the periphery much faster than they multiply and become fewer as the radius of the circlelike lesion, the center of which is the point of inoculation, becomes longer. In consequence of this the lesion narrows down to a thinner and thinner line, the farther toward the periphery the lesion spreads. This progress in a regular circle of the treponemas is modified when they invade the skin of the root of the nose. Leaving out the eyelids, they spread down the side of the nose in a butterfly effect. In one instance an experimental animal was inoculated on the eyebrow and, due to the admixture of pyogenic bacteria with the inoculum, an erysipelatous infection set in. It extended over the entire eyebrows and down the root of the nose, and finally healed. No yaws lesion developed on

the eyebrows of this animal but, after an unusually long incubation, a typical yaws lesion developed below the right eye. This is the only case among our inoculated animals that developed a yaws lesion at some distance from the original point of inoculation, but the lesion developed in a place that is on the route by which the spreading lesion always progresses from the eyebrow down the side of the nose. It clearly indicates that treponemas travel or are carried through lymphatic spaces and thus naturally reach the first filter, the regional lymph-gland (see lymphogenic metastases).

The observation on healed and cured monkeys with regard to the relapses has shown that not a single one of the healed or cured monkeys developed a relapse of clinical manifestations, although some of the observations were extended over a period of three years. If we examine the results of our observations on other phases of experimental yaws infection, as given elsewhere in this paper, we can see in the local exacerbation of a spreading or healing local yaw the innate tendency of treponematous infections toward relapses or exacerbations well pronounced. In that respect, therefore, the monkey organism behaves very similar to that of man where likewise periods of lull followed by a period of exacerbation in an existing lesion can be clearly discerned. However, the fact that no relapse was noticed among our numerous experimental animals is not to be attributed to the difference in behavior of the body organism of the monkey from that of man. As long as the lesion exists and before the immunity has developed to a sufficient degree to prevent, the local exacerbations and metastatic lesions may be expected, in monkey as well as in man. The absolute lack of relapses following the disappearance of the clinical lesion must therefore be interpreted in the following way: The immunity that has developed in the meantime prevents the treponemas existing in the lesion from metastatic spreading toward the other parts of the body and, following the healing of all skin lesions, there is in yaws no permanent deposition of treponemas in the lymphatic apparatus or elsewhere from which the relapses might originate. The immunity develops rapidly enough and is so lasting that the period of the metastatic dissemination of *Treponema pertenue*, in monkeys in particular, is limited. Even though they may, at the time of metastatic distribution, reach other organs than the skin they do not, according to their nature, gain a foothold in these organs. The typical metastatic lesions

that have been described in this paper cannot be explained in any other way than as originating through hæmatogenic dissemination.

From our study of the yaws infection in Philippine monkeys the only possibility of latent infection followed by relapses would be as in the following coincidence. We noticed that between the third and the fifth month of infection and due to superinfection a generalization of yaws may take place. This generalization is heralded by very intensive local exacerbation of the existing lesion. It is at this time, when the exacerbation takes place in an eruptive manner, that the hæmatogenic distribution of the treponemas takes place. We have noticed that, following the occurrence of intensive local exacerbation, the generalized skin manifestations, if they occur at all, will be noticed within about four or six weeks from the time of the exacerbation. It must be presumed, from this time relation between the occurrence of the generalized skin manifestation and the occurrence of the local exacerbation, that the actual dissemination of the treponemas happens simultaneously with the local exacerbations. The time between these two phenomena agrees well with the incubation of a local yaw; that is, the period between the time of inoculation and the time when the local lesion assumes a clinical form of beginning yaw. If it so happened that the exacerbated local yaw would heal completely before the incubation period of the metastatic yaws has elapsed, we should have a short period of latent infection in yaws where the animal, or the man for that matter, shows no evident lesions but in due time develops a crop of metastatic yaws.

Although the incubation of the primary yaw in a normal individual man or monkey is fairly constant, yet we have seen in human volunteers that the incubation of a reinoculation may be shortened or lengthened.(29) Similar prolongation of the incubation period was noticed in monkeys in sporadic cases; the shortest observed was sixteen days, and the longest was fifty-two days. Therefore, the theoretical possibility of a latent infection in yaws exists, and the organ in which the treponemas remain dormant for longer than twenty-six days is the skin. Therefore, we may claim that in yaws the latency is theoretically possible but very unlikely, on account of the fact that the mother yaw usually remains active up to the time when the generalized distribution occurs, or even longer. The latency,

as we interpret it (that is, latency followed by relapses), is determined by the time relation between the healing of the lesions and the duration of incubation of metastatic yaws. The quicker the healing and the longer the incubation the longer will be the period of latency. Once the lesions are completely healed and the maximum incubation period has expired, without the occurrence of relapses, the further propagation of the yaws process becomes impossible.

Therefore, no evidence was found in our observations that relapses would occur in animals with the local, the local and generalized yaws, or the local and late ulcerative form of yaws (see Table 16).

The microscopic examination of the lymph glands by means of dark-field illumination resulted in negative findings, not only in animals with healed or cured yaws, but also in animals with active yaws. This observation entitles us to only one conclusion; that is, that in any case of experimental yaws treponemas in the regional lymphatic node, if present at all, are present in far smaller numbers and they do not multiply there to such an extent as they do in the corresponding skin lesion.

Table 17 shows the results of our inoculation experiments.

TABLE 16.—*Showing the results of clinical observations of healed yaws in monkeys with respect to relapses.*

Number of monkeys used.	Clinical form of yaws.	Duration of observation.	Relapses occurred in—
		Months.	
Two	Local	1	0
Two	do	2	0
One	do	3	0
One	Generalized	3	0
Five	Local	4	0
One	do	5	0
Two	do	6	0
One	Generalized	9	0
Four	Local	9	0
Five	do	10	0
One	Generalized	10	0
Three	Local	11	0
Two	do	12	0
One	do	14	0
One	do	15	0
One	do	16	0
One	do	19	0
One	do	23	0

TABLE 17.—*Showing the results of lymph gland inoculations in Philippine monkeys.*

[+, positive; —, negative; tr, treated with neosalvarsan.]

Recipient monkey.	Lesion.			Treponemas in lesion.	Result.		Donor monkey.
	Active.	Healing.	Healed.		Microscopic.	Take.	
R-2	+	—	—	+	—	—	T-13
R-2	+	—	—	+	—	—	Y-2
R-2	+	—	—	+	—	—	H-21
K-7	+	—	—	+	—	+	J-15
A-6	+	+	—	+	—	+	B-5
P-14	+	—	—	+	—	—	A-7
N-15	+	+	—	+	—	—	B-5
L-7	—	+	—	+	—	+	G-9
Y-5	+	+	—	+	—	—	A-6
Y-5	+	—	—	+	—	—	O-d
Y-5	—	—	+	—	—	—	B-6
Y-4	—	—	+	—	—	—	P-13
Y-4	—	—	+	—	—	—	A-7
Y-4	+	—	—	+	—	—	G-10
Y-6	+ late	+	—	—	—	—	H-18
Y-6	+ tr	—	—	+	—	—	Baby I
Y-3	—	—	+ tr	—	—	—	B-K-3
Y-3	—	—	+ tr	—	—	—	B-K-2

The procedure followed in this investigation was to remove the corresponding regional gland (that is, the inguinal gland) surgically and aseptically. The gland was triturated in a sterile mortar and a small amount of salt solution added. The emulsion of practically the entire gland was taken up in a small hypodermic syringe and injected intradermally, following the method indicated in the beginning of this paper. The remnant of the emulsion was used for microscopic dark-field examination.

We encountered no difficulty in locating the healed lesion. Besides our records, we used as a guide the persistent pigmentation of the scrotum that remained for a long time after the healing of the lesion. From Table 17 it is evident that the injection of the emulsified lymph gland produced a yaws lesion in only a few instances. These were glands taken from monkeys with active lesions. However, several inoculations of glands from monkeys with active lesions failed to produce yaws lesions, and not a single inoculation of lymph glands from monkeys with healed or cured yaws produced yaws lesions. From this negative evidence, combined with the results of our microscopic examinations and the results of observations on relapses in cured or healed yaws monkeys, we believe we are justified

in claiming that the invasion of treponemas into the lymphatic apparatus of experimental yaws monkeys has no significance with regard to the interpretation of latent infection. It would not be surprising to find that the treponemas survive in the lymph glands after the corresponding lesion has healed. If this be the case an explanation other than latent infection would be more justified. We find that active yaws lesions last at times for months after the onset of the resistance to reinoculation, and it is the developed immunity that prevents further metastatic spreading of the yaws process to other parts of the body. The treponemas remain at the place of the lesion until the healing process has removed them entirely.

That this interpretation is correct is brought out by the observation on animals and man who developed late ulcerative yaws lesions without going through the generalized metastatic stage. This condition of body shows a long-protracted period of reinoculability and also long-delayed healing of the ulcerative lesion. In spite of the fact that the healing is protracted in the cases of ulcerative lesions, the animals, as is the case in man, do not develop further generalization even though a focus has been established by the development of the chronic lesion in which treponemas, evidently in small numbers, maintained themselves for months. Partial immunity has developed by that time, which is sufficient to prevent the generalization, but not sufficient to prevent development of an atypical yaw as a consequence of superinfection.

In this connection the question necessarily arises whether the resistance to inoculation of yaws, as evident in experiments on animals and humans, is a sign of immunity or a sign of latent infection, as claimed by some. With regard to yaws, whatever the condition in other treponematous diseases may be, at least as far as animals are concerned, no evidence of latent infection has been noticed in our experimental observations extending over more than three years. Unless a spontaneous eruption of yaws is noticed in animals that have been perfectly free from any manifestations or symptoms of yaws due to thorough treatment or spontaneous recovery, it would be fallacy to speak of latent infection, since there is neither indication nor sign of such.

If we consider as a working basis the argument that latent infection is responsible for the resistance to reinoculation we should expect that, in experimental cases of late ulcerative, so-called "tertiary" lesions of long duration, the resistance to reinoc-

ulation would be much greater than in cases that have run a rapid and extensive course characterized by repeated crops of yaws of short duration. In looking over our tabulated results and the results of experiments on late human cases of yaws,(29) we see that quite the opposite is the case. In the first place, not a single one of the generalized cases of yaws in monkeys developed so-called "tertiary" lesions, but they proved most resistant to superinfection and developed this resistance in a very short time. On the other hand, those animals that after a long course of mild local yaw developed so-called "tertiary" lesions were repeatedly reinoculable, and their resistance did not become marked until after long persistence of the tertiary lesions. These findings cannot be brought into harmony with the theory that latent infection is responsible for the resistance to superinoculation. Once the duration of yaws infection, in patients and in animals alike, has extended over the border line of reinoculability, treatment brings about a rapid healing of the lesion but has no effect whatsoever on their resistance to reinoculation.

Evidence is on hand that Philippine monkeys are more resistant to yaws than are humans. At least, with regard to the generalization, the statement is unquestionably true. It is furthermore an established fact that resistance to superinfection is more nearly complete and develops much earlier in the generalized stage of yaws in monkeys than it does in man. It is hardly conceivable that "latent infection" should develop more quickly and to a higher degree in a resistant monkey than in a susceptible man.

The failure to realize the independence of healing of treponematous lesions from the development of resistance to superinfection or reinfection led to the theory that latent infection is the cause of the resistance. It is confusing the cause with the effect. A similar condition, where immunity brings about a localization of infection without in any way injuring the microörganism, is not unknown in bacterial immunity. One instance is the immunity following active immunization with aggresins against symptomatic anthrax.(27)

It is obvious from this that reinoculation cannot be used as a criterion for complete cure or sterilization of the body organism of either man or animal.

First of all the man, like the animal, can be superinfected while the infection exists in a form of local yaws (for six months), in a form of generalized yaws as long as the metas-

tatic dissemination continues, and in a late ulcerative form practically during the entire duration of the active ulcerative lesion.

On the other hand, superinfection is not successful after the seventh month, while evident lesions persist for months and treponemas persist in the lesions for a long time after the body organism became completely resistant to either artificial superinfection or to spontaneous generalization.

The occasional presence in small numbers of *Treponema pertenue* within the regional glands indicates the way by which the treponemas travel and in certain cases, particularly as a consequence of superinfection, produce generalization. In a great majority of experimental cases, however, they are halted by the first barrier, the corresponding lymph gland, and perish then and there. Thus they are prevented from reaching their goal, the skin, the only organ where they produce characteristic lesions and can persist for a long time, particularly in the late so-called "tertiary" stage.

The periodic broadcasting of the treponemas into the blood stream, followed by cropping out of metastatic lesions on the skin, is brought to a standstill by the immunity which localizes the infection in the lesions that have already developed and prevents further metastatic spreading. Consequently, the period of the appearance of the metastatic yaws lesion is limited and its duration dependent upon the onset of immunity. Likewise, the partial immunity that develops in the animals with late so-called "tertiary" lesions is responsible for the finding that, once an ulcerative lesion has developed on the skin, although the normal skin of the same animal is still susceptible to development of an atypical yaws lesion, no generalization takes place. The character of immunity may, therefore, be defined as an ability on the part of the body to localize the infection and, although the existing lesion may spread by continuity, no new metastatic lesions develop. In this respect the immunity resembles very much the antiaggressive immunity as known in certain bacterial infection and, again, the importance of the lymphatic apparatus in the destruction of the treponemas is brought into view.

SIGNIFICANCE OF EXPERIMENTAL FINDINGS PRESENTED

Aside from the weight of proof of the experimental evidence brought forth in this investigation with regard to the etiology

and the pathogenesis of conditions such as gangosa, keratoderma plantare, psoriasis palmaris, and other early evanescent frambœsides, these experimental findings have a great significance with regard to the interpretation of immunity in yaws. The subject of relapses in yaws has been loosely discussed from a clinical standpoint. As a rule, a case of yaws that again develops frambœsia after treatment is considered a relapse. The clinical cases of yaws are ambulatory patients and not cases under strict control. The possibility of reinfection is generally not seriously considered by the clinicians and epidemiologists. To those acquainted with syphilis only, the phenomenon of relapses in yaws appears to be a natural thing. In the light of present knowledge, gained by recent experiments on animals and humans, the question of relapses and reinfection in yaws patients must be considered more carefully than has been done hitherto.

With the experimental evidence presented in our researches, we can explain and understand certain disagreements in experimental infection and reinfection performed on humans. The irregular results of reinoculation of yaws patients, as published in the literature, could not heretofore be placed in any comprehensible system.

Omitting the discussion of experiments of this kind on humans performed elsewhere, because of lack of acquaintance with the details, I shall discuss only those of my own experience that have been performed during the last sixteen years in the Philippines.

Very illustrative experiments were those of Sellards and Goodpasture(29) and of Sellards, Lacy, and Schöbl.(30) Briefly stated, the experiments were as follows:

In 1922 and six months after treatment with neosalvarsan four patients were inoculated with yaws. Two of the four cases developed lesions of sufficient extent to require treatment with neosalvarsan. After an interval of more than two years the susceptibility of these four patients to yaws was tested again by reinoculation. They had received no further treatment in the meantime.

The result of this and subsequent reinoculations was as follows:

Upon first reinoculation patients 1 and 4 showed lesions recognizable as yaws. While No. 1 developed early generalized lesions after five weeks, No. 4 failed to develop them after four months. Nos. 2 and 3 exhibited no lesion at the place

of inoculation recognizable as yaws. Another treatment with neosalvarsan was given and the same four patients were reinoculated with the following result: No. 1 again developed a typical granuloma and generalized lesions, while No. 4, like Nos. 2 and 3, remained negative as far as yaws lesions were concerned.

These results are rather confusing and, at the time the researches were finished, were not quite comprehensible; but, with the accumulated experimental evidence with regard to immunity to yaws in animals, the reason can be seen at once for the different behavior of the four patients, all of whom in the beginning and at the time of the first treatment presented fully developed, generalized, so-called "secondary" yaws. The explanation lies in Table 1 of Lacy and Sellards.(12) It is evident that at the time the first treatment was given the infection with yaws had lasted in patient 1 only three months and in patient 4 only eight months, while in patients 2 and 3 it had lasted twelve and twenty-four months, respectively. This shows clearly that if the infection in man, as in animals, has lasted less than seven months the patient or the animal is reinoculable. If, however, the infection has lasted longer than eight months the patient or the animal is no longer reinoculable. While patient 1 was found repeatedly reinoculable when treatment was given after the first, and the second infection lasted less than seven months and therefore he was not immune, patient 4 was at the time of the first treatment just on the border line of reinoculability and therefore gave a positive take (local only) upon the first reinoculation and a negative take on the second reinoculation. Patients 2 and 3 had their natural infection for a sufficiently long time previous to the first treatment to develop their immunity and therefore were not reinoculable, in spite of treatment. This interpretation of the experimental evidence makes the clinical assumption, which lacks all experimental evidence, of relapses questionable. By relapses in this discussion, it is to be strictly understood, are meant the occurrence of yaws in one and the same patient after all of the clinical lesions and symptoms have completely disappeared due to healing or treatment, and does not refer to exacerbation of the existing or vanishing yaws process.

Summarizing briefly, we should state that a patient, like the experimental animal, that went through a typical course of generalized yaws and after the infection had lasted for more than eight months, either healed spontaneously or was treated to such

an extent that all of the specific and nonspecific manifestations of yaws had disappeared, can neither be reinoculated nor contract yaws again.

With regard to the question how long this desirable condition on the part of the patient lasts, we are still in the dark, but it can be reasonably assumed that, in case the patient has gone through typical and repeated crops of generalized yaws within the period of time indicated above, his resistance will last for a long time, if not for life. The fact that the majority of the yaws cases in a given infested locality are children and youths, while the adults are free from yaws manifestations, finds its explanation in our experimental investigation. The experiments of Sellards, Lacy, and Schöbl(30) on humans show that the resistance lasts more than two years.

Objections will naturally be raised, and the question will be asked, How can this interpretation explain the existence of late yaws manifestations in old people? Unfortunately, in analogy to syphilis, these lesions are generally considered by exclusive clinicians as relapses of latent yaws. Whether or not this interpretation is correct with regard to syphilis, experimental evidence being lacking, is an open question; but it certainly is not true in cases of yaws. We must bear in mind that frambœsia tropica is primarily and in the majority of cases exclusively a skin affection, and that spontaneous healing and therapeutic cure are far more rapid and complete in yaws than in syphilis. Furthermore, a permanent localization of *Treponema pertenue* in the internal organs has not been demonstrated satisfactorily. It certainly is not the rule. It is an open question whether or not the bone lesions in yaws, either early or late, are always due to actual localization of *Treponema pertenue* in the bones or whether it is a process analogous to that of keratoderma plantare.

Furthermore, it must be remembered that ulcerative so-called "tertiary" lesions do not occur metastatically as such, but develop from existing early lesions or (in experimental animals, at least) it has been demonstrated that they occur as a result of superinfection which took place during the second allergic stage. The explanation therefore for the occurrence of late ulcerative lesions of long duration, based on experimental evidence, would be a low-grade infection caused by low-grade reactivity of the body organism toward the infection, which reactivity manifests itself by the negative phase and the con-

sequent occurrence of a sufficient number of metastatic early lesions. Indeed, in late ulcerative cases giving no history whatsoever of early metastatic lesions, so-called "secondaries" are probably the rule rather than the exception. Restricting again our quotation of literature to the work done in the Philippines we find the description of four cases of late ulcerative yaws lesions reported by Ferdinand Schmitter.(26) The author says: "The history in each of the above four cases was of an extragenital papule without secondaries."

Sufficient evidence has been produced that the cases of late yaws in experimental animals are reinoculable, a finding which completely agrees with the experience of Sellards and Goodpasture(29) who, experimenting on cases of "clavos," arrived at the same conclusion with regard to humans. The patients, like the experimental animals, who develop late ulcerative so-called "tertiary" lesions, have not gone through crops of generalized yaws manifestations at all, or not a sufficient number of them to develop such a degree of immunity as is conferred on patients who went through a typical initial yaw and had repeated crops of generalized manifestations.

The place of predilection of ulcerative yaws lesions in many patients remarkably coincides with the predilection place of the mother yaw, and the initial yaw not infrequently turns into an ulcerative chronic lesion—so frequently, in fact, that the initial lesion of yaws is given in texbooks erroneously as an ulcer.

The resistance as it develops during the course of generalized yaws in monkeys, and for that matter in man, has all the characteristics of immunity. In the beginning, when the local yaw only develops, the animals are as susceptible to superinfection as are normal animals. Then follows a state of higher susceptibility, a negative phase, manifesting itself by an intensive local process, enormous numbers of treponemas in the lesion, and generalization of the process. This stage is followed by increased resistance to superinfection that becomes complete in due time, is lasting, and is independent of treatment.

CONCLUSIONS

1. The Philippine monkey is an excellent experimental animal, due to its high susceptibility to yaws and on account of the variety of clinical lesions that can be produced experimentally in this animal.

2. The local lesion produced by intradermal inoculation of Philippine monkeys is a yaw clinically and anatomically identical with that produced in human volunteers experimentally.

3. The early metastatic yaws lesions produced in Philippine monkeys by superinfection—that is, the typical metastatic yaw, the ringworm yaw, the early frambœsides including psoriasis palmaris—are clinically and anatomically identical with metastatic manifestations of yaws in humans.

4. The late yaws lesions, such as the ulcerative form, lupus-like lesions, gangosa, and the late frambœsides, such as ichthyotic yaws lesions and the keratoderma plantare as produced in monkeys by superinfection, are clinically and anatomically identical with these lesions as they occur in man.

5. The duration of incubation of local yaw is the same in Philippine monkeys as it has been established to be in human volunteers.

6. The incubation of the metastatic generalization of yaws produced in Philippine monkeys by superinoculation is the same as that found in human volunteers upon experimental inoculation.

7. The duration of early yaws manifestations, as well as that of the late ones, is much shorter in Philippine monkeys than is found by clinical experience to be the case in man.

8. However, the proportion of the duration of early yaws manifestations to the duration of late yaws manifestations is about the same in monkeys as in man.

9. The immunity, which consists of resistance to superinfection and resistance to metastatic generalization as well as of modification of the early and late lesions that take place at the time when the resistance to superinfection starts to develop, set in with Philippine monkeys much earlier than was found to be the case in experimentally inoculated human volunteers.

10. The fact that the period of metastatic dissemination of yaws is much more limited in monkeys than in man is due to the early onset of immunity.

11. The healing of existing yaws lesions, particularly the early ones, is independent of the resistance to superinfection. Yaws lesions in monkeys, as in man, may heal while the animal or the man is still susceptible to superinfection, and existing lesions will persist a long time after the stage of resistance to superinoculation has fully developed.

12. From this it is evident that the reinoculability of yaws animals cannot be used as a criterion for complete therapeutic sterilization of the yaws-infected body organism.

13. The resistance to superinfection once achieved is persistent, and no amount of treatment can cause the animal, once it has become resistant, to take infection again.

14. The Wassermann reaction is indefinite and ephemeral in the case of local yaws. Its strength and persistence depend upon the duration of infection, the number of yaws lesions, the intensity of the lesion and, to a lesser extent, on the number of superinoculations.

15. The Wassermann reaction, if it has become negative due to treatment or spontaneous healing and if all the lesions have disappeared, will reappear upon unsuccessful superinfection or reinoculation with viable material.

16. The serologic reactivity of the body organism to superinfection (that is, the reappearance of the Wassermann reaction and the reactivity of the organism to treatment, which manifests itself as a disappearance of the Wassermann reaction) becomes sluggish upon repeated reinoculations and treatment.

17. The reappearance of a positive Wassermann reaction can be produced in healed and cured animals without recurrence of yaws lesions and, therefore, a positive Wassermann reaction does not necessarily mean the existence of *Treponema pertenue* in the body organism of the animal.

18. The focus from which the treponemas are disseminated into the surrounding tissues, or metastatically into remote parts of the body, is the skin.

19. In lymph glands corresponding to the active lesions *Treponema pertenue* can be found in a fairly high percentage of cases while the early lesion is active, but *Treponema pertenue* was never found in the lymph gland when the lesion had healed, either spontaneously or due to treatment.

20. Spontaneous relapses do not occur in experimental monkeys when they reach the stage of resistance. The temporary stay of *Treponema pertenue* in the regional lymp gland indicates the route through which generalization in yaws takes place, but it has no significance with regard to possible relapses after a period of latency.

21. The latency in yaws followed by relapse depends upon the time relation between the healing of the existing yaws lesions and the incubation period of the metastatic yaws.

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SCHEMATIC PROTOCOLS OF SUPERINFECTED AND REINFECTED MONKEYS, ARRANGED ALPHABETICALLY IN GROUPS

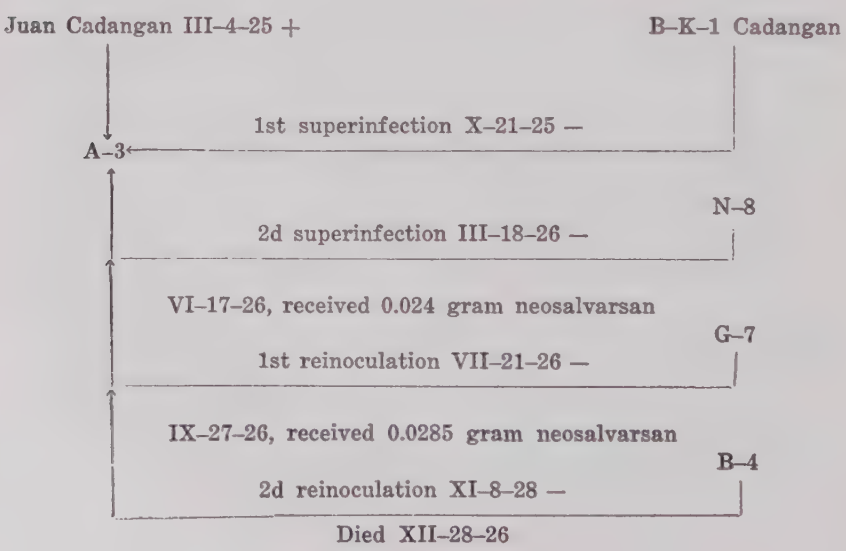
I. Monkeys with local yaws.
II. Monkeys with generalized yaws.
III. Monkeys with late ulcerative yaws and other late forms.
IV. Monkeys reinoculated after treatment.

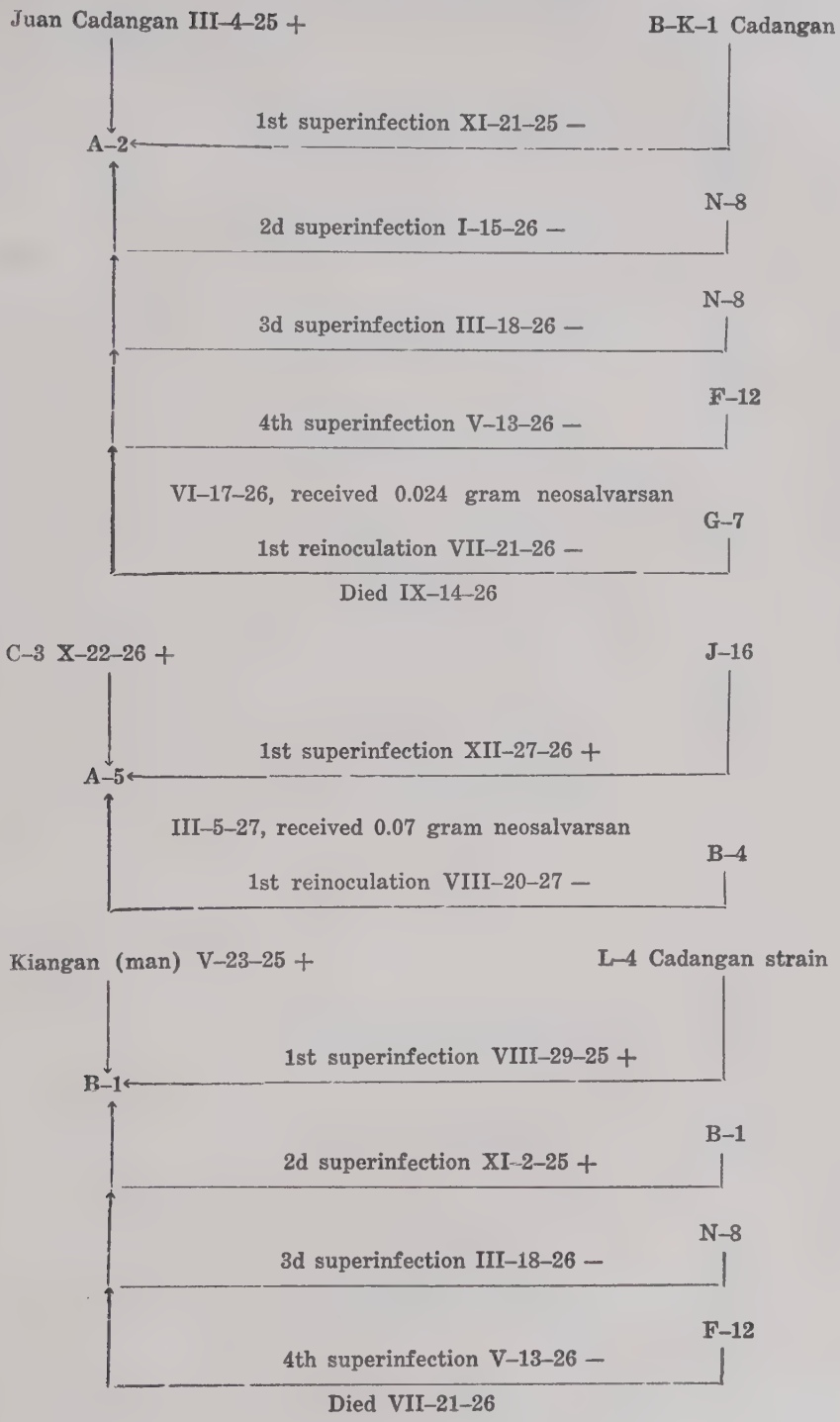
The protocols of monkeys that were used for superinfection first and then, seven or more months later, for reinoculation, are listed in groups I, II, and III.

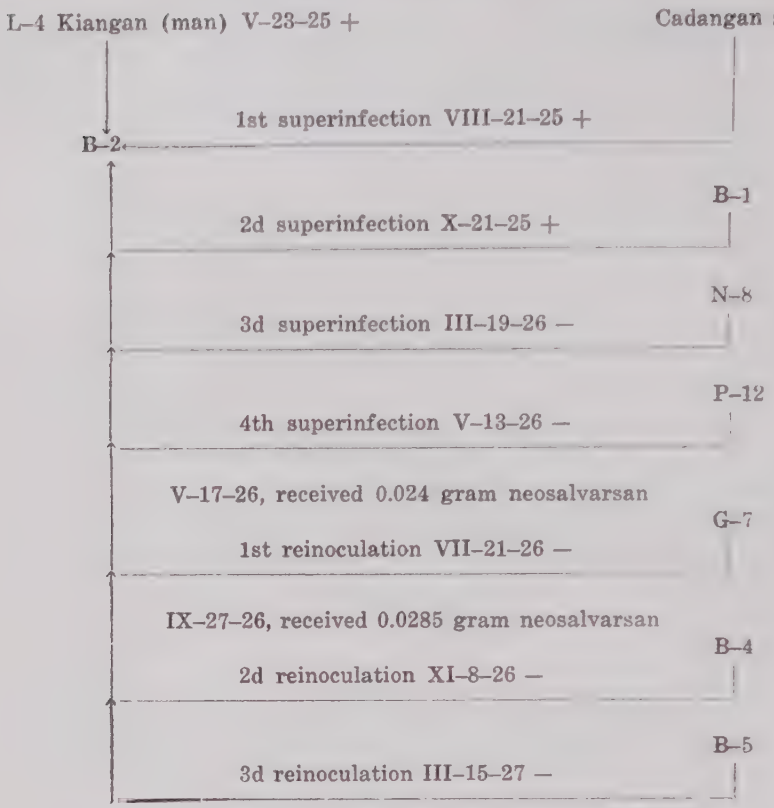
Donor ⟶ Recipient

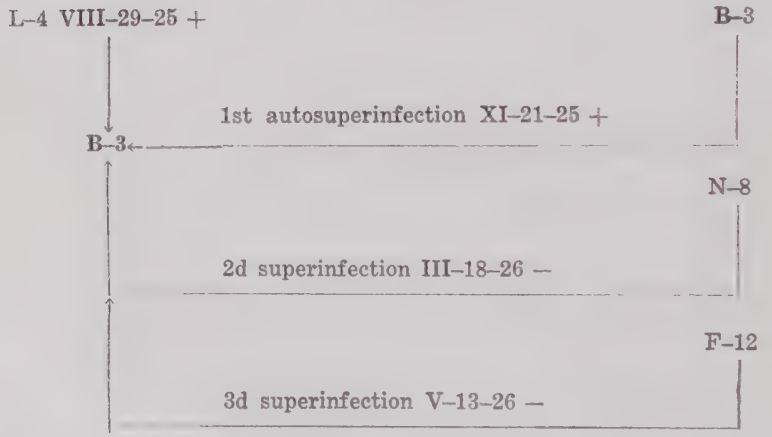
For the sake of easier calculation the months are indicated by Roman numerals, the days and years by Arabic figures. +, positive take; ±, feeble take; —, negative take.

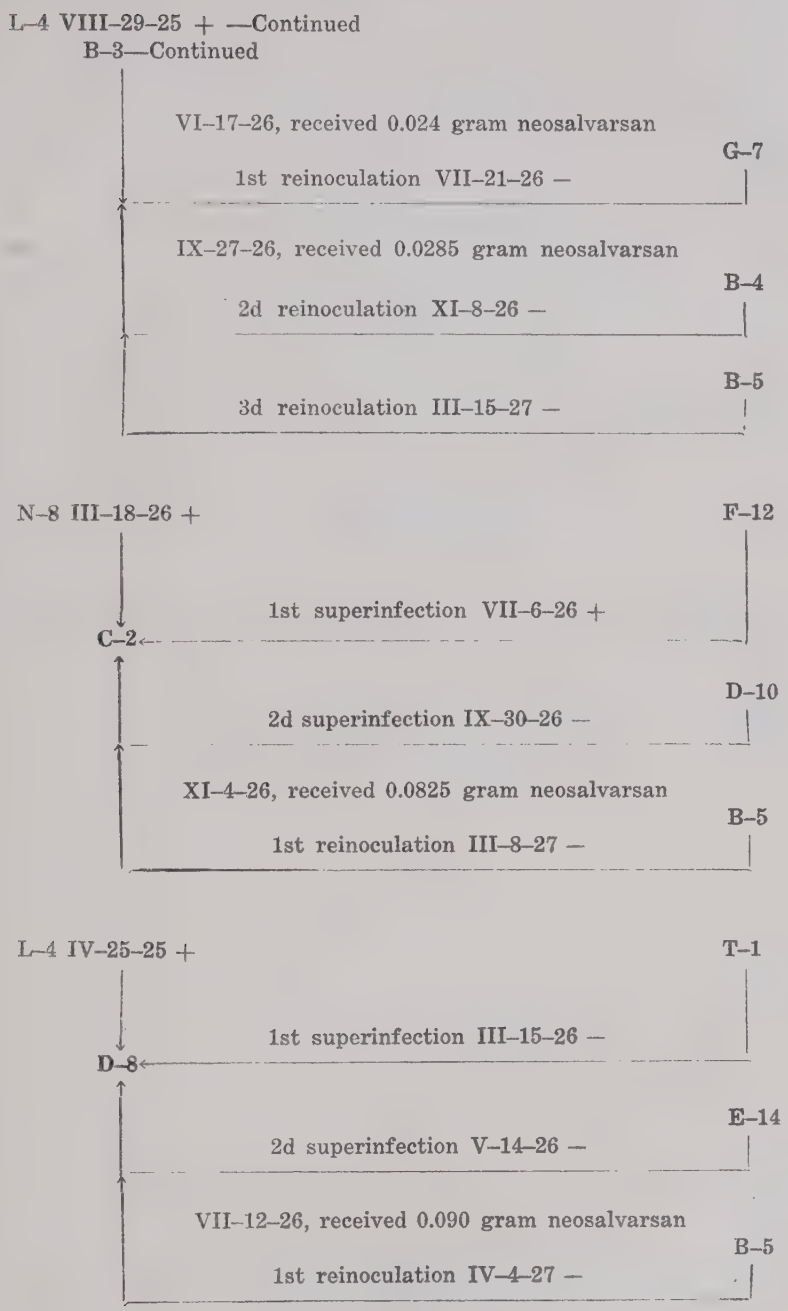
GROUP I. SUPERINFECTION AND REINOCULATION OF POSITIVE YAWS MONKEYS

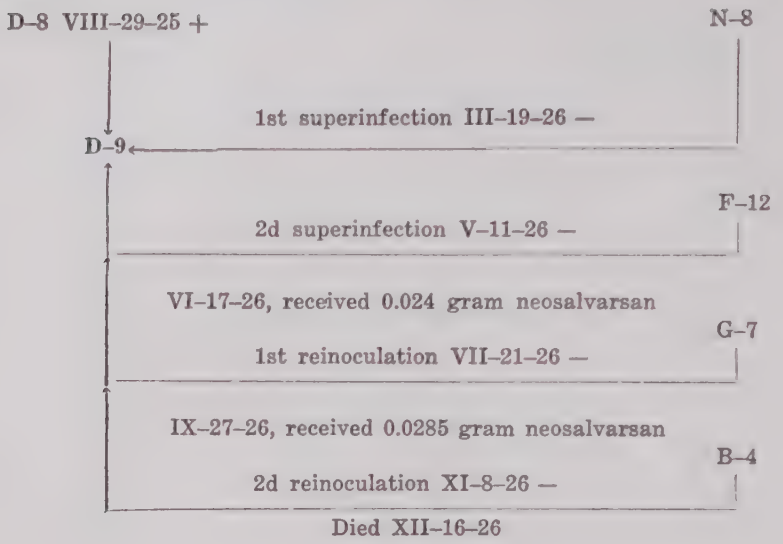


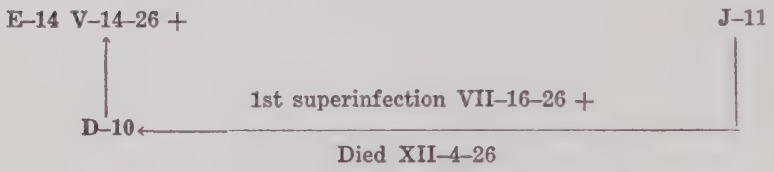

Juan Cadangan III–4–25 +
B–K–1 Cadangan
1st superinfection XI–21–25 —
A–2
N–8
2d superinfection I–15–26 —
N–8
3d superinfection III–18–26 —
F–12
4th superinfection V–13–26 —
VI–17–26, received 0.024 gram neosalvarsan
G–7
1st reinoculation VII–21–26 —
Died IX–14–26
C–3 X–22–26 +
J–16
1st superinfection XII–27–26 +
A–5
III–5–27, received 0.07 gram neosalvarsan
B–4
1st reinoculation VIII–20–27 —
Kiangan (man) V–23–25 +
L–4 Cadangan strain
1st superinfection VIII–29–25 +
B–1
B–1
2d superinfection XI–2–25 +
N–8
3d superinfection III–18–26 —
F–12
4th superinfection V–13–26 —
Died VII–21–26

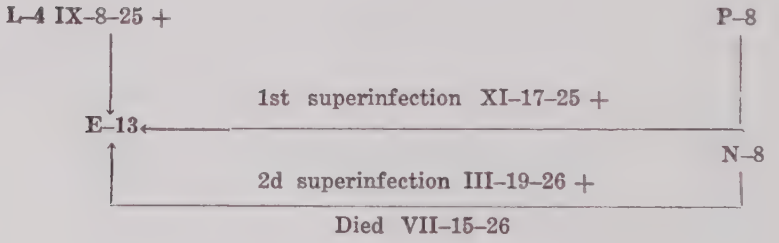

L–4 Kiangan (man) V–23–25 +
Cadangan strain
1st superinfection VIII–21–25 +
B–2
B–1
2d superinfection X–21–25 +
N–8
3d superinfection III–19–26 –
P–12
4th superinfection V–13–26 –
V–17–26, received 0.024 gram neosalvarsan
G–7
1st reinoculation VII–21–26 –
IX–27–26, received 0.0285 gram neosalvarsan
B–4
2d reinoculation XI–8–26 –
B–5
3d reinoculation III–15–27 –

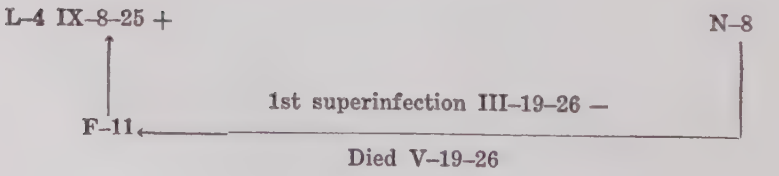

L–4 VIII–29–25 +
B–3
1st autosuperinfection XI–21–25 +
B–3
N–8
2d superinfection III–18–26 –
F–12
3d superinfection V–13–26 –

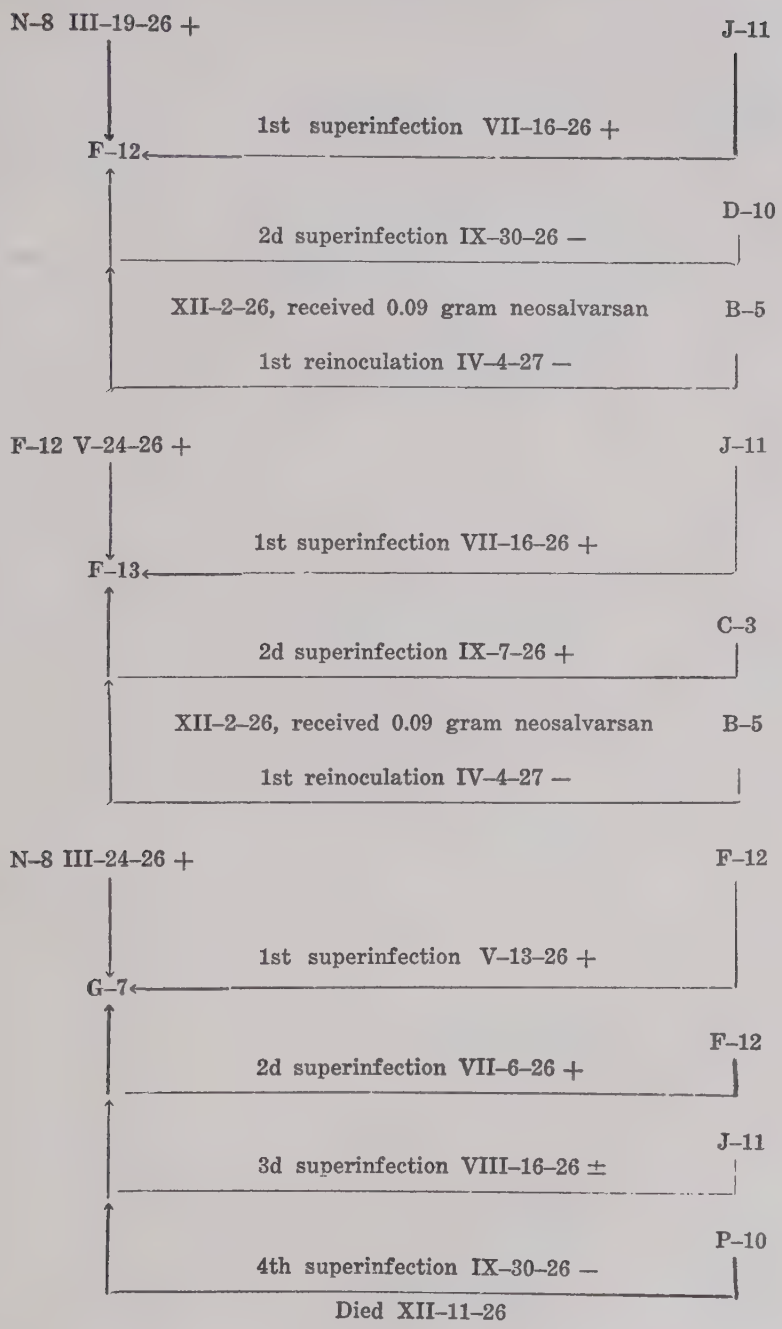

L–4 VIII–29–25 + —Continued
B–3—Continued
VI–17–26, received 0.024 gram neosalvarsan
G–7
1st reinoculation VII–21–26 —
IX–27–26, received 0.0285 gram neosalvarsan
B–4
2d reinoculation XI–8–26 —
B–5
3d reinoculation III–15–27 —
N–8 III–18–26 +
F–12
1st superinfection VII–6–26 +
C–2
D–10
2d superinfection IX–30–26 —
XI–4–26, received 0.0825 gram neosalvarsan
B–5
1st reinoculation III–8–27 —
L–4 IV–25–25 +
T–1
1st superinfection III–15–26 —
D–8
E–14
2d superinfection V–14–26 —
VII–12–26, received 0.090 gram neosalvarsan
B–5
1st reinoculation IV–4–27 —

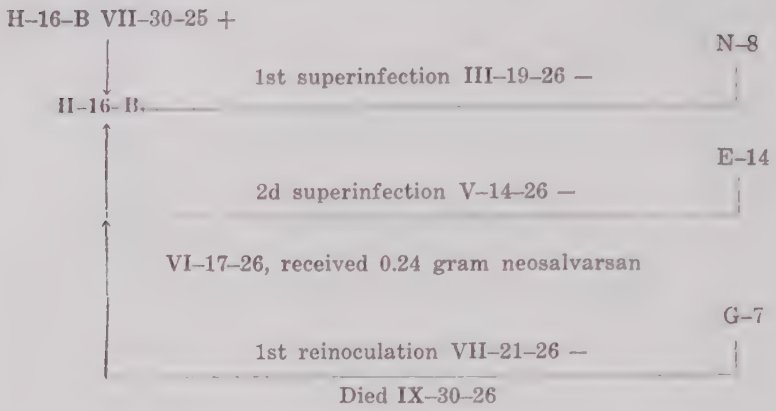

D–8 VIII–29–25 +
N–8
1st superinfection III–19–26 –
D–9
F–12
2d superinfection V–11–26 –
VI–17–26, received 0.024 gram neosalvarsan
G–7
1st reinoculation VII–21–26 –
IX–27–26, received 0.0285 gram neosalvarsan
B–4
2d reinoculation XI–8–26 –
Died XII–16–26

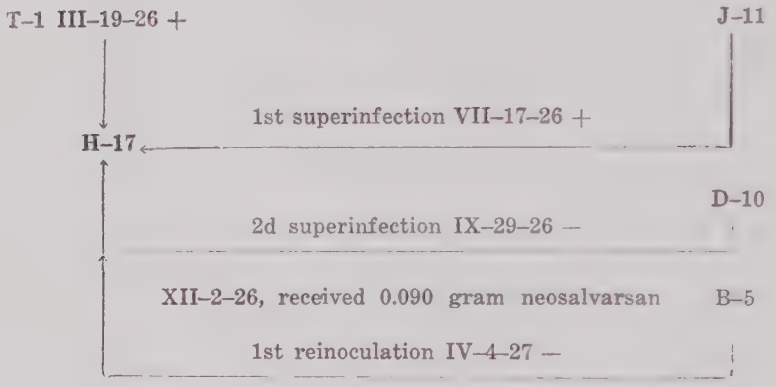

E–14 V–14–26 +
J–11
1st superinfection VII–16–26 +
D–10
Died XII–4–26

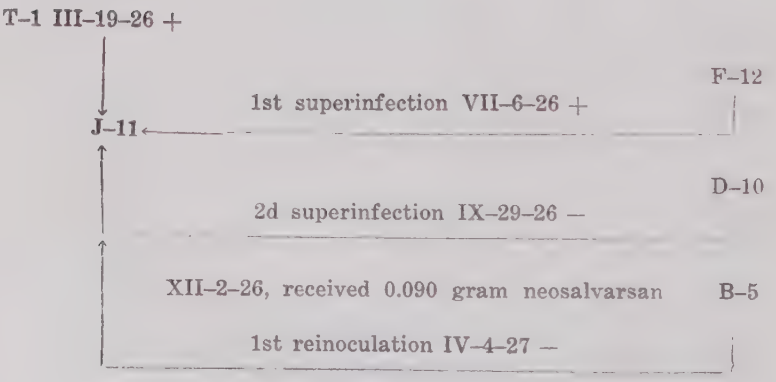

L–4 IX–8–25 +
P–8
1st superinfection XI–17–25 +
E–13
N–8
2d superinfection III–19–26 +
Died VII–15–26

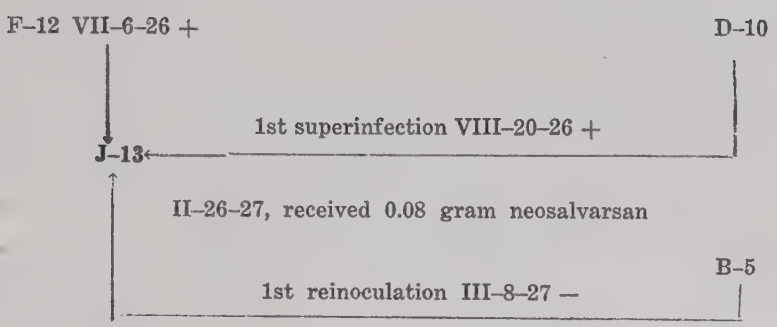

L–4 IX–8–25 +
N–8
1st superinfection III–19–26 –
F–11
Died V–19–26

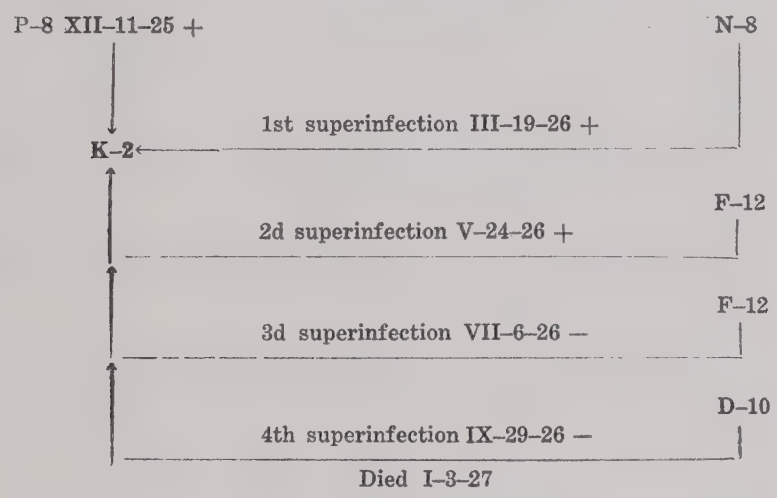

N–8 III–19–26 +
J–11
1st superinfection VII–16–26 +
F–12
D–10
2d superinfection IX–30–26 —
XII–2–26, received 0.09 gram neosalvarsan
B–5
1st reinoculation IV–4–27 —
F–12 V–24–26 +
J–11
1st superinfection VII–16–26 +
F–13
C–3
2d superinfection IX–7–26 +
XII–2–26, received 0.09 gram neosalvarsan
B–5
1st reinoculation IV–4–27 —
N–8 III–24–26 +
F–12
1st superinfection V–13–26 +
G–7
F–12
2d superinfection VII–6–26 +
J–11
3d superinfection VIII–16–26 ±
P–10
4th superinfection IX–30–26 —
Died XII–11–26

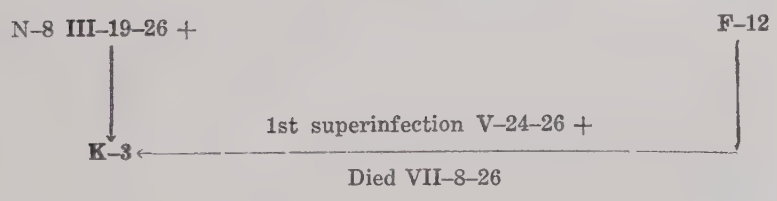

H–16–B VII–30–25 +
N–8
1st superinfection III–19–26 –
H–16–B
E–14
2d superinfection V–14–26 –
VI–17–26, received 0.24 gram neosalvarsan
G–7
1st reinoculation VII–21–26 –
Died IX–30–26

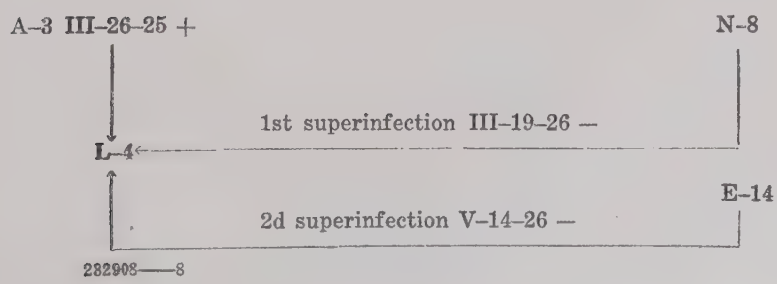

T–1 III–19–26 +
J–11
1st superinfection VII–17–26 +
H–17
D–10
2d superinfection IX–29–26 –
XII–2–26, received 0.090 gram neosalvarsan
B–5
1st reinoculation IV–4–27 –

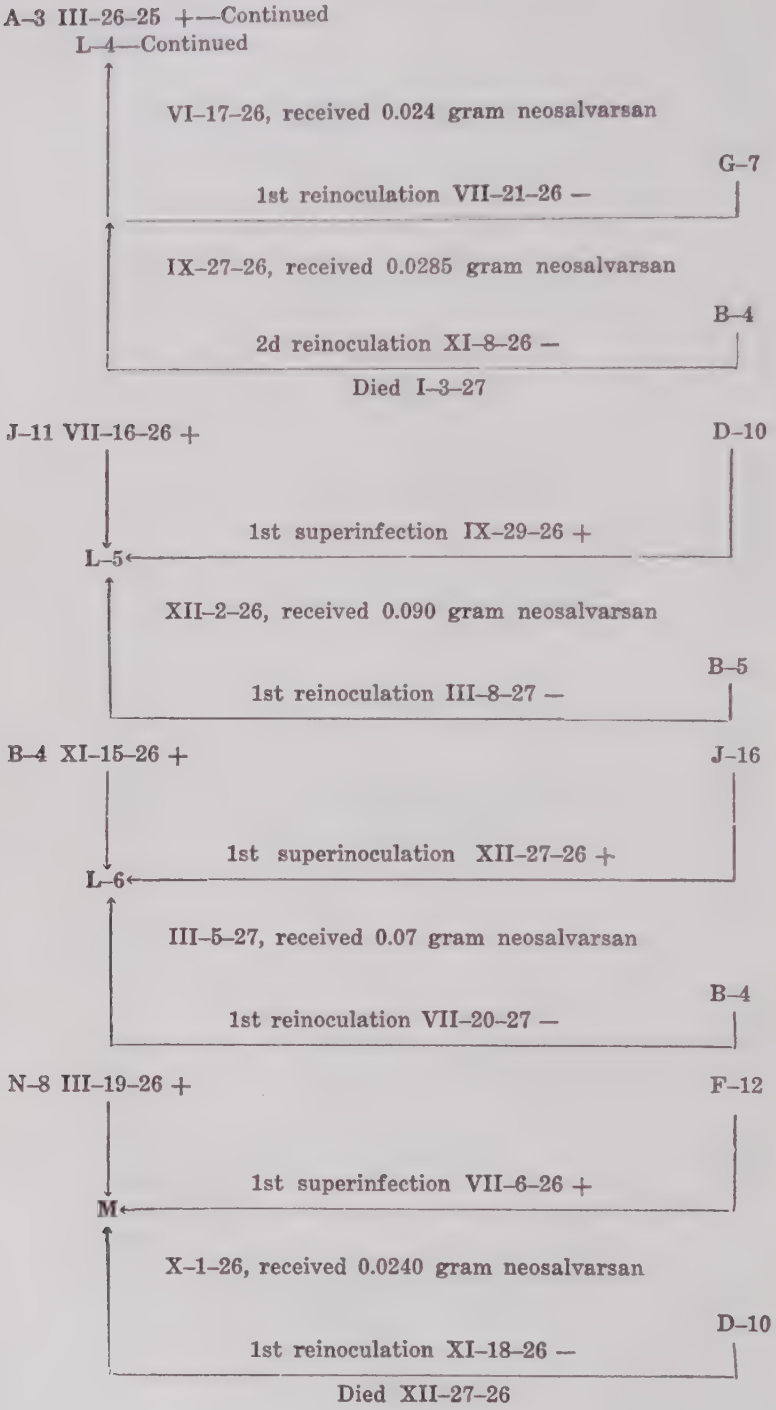

T–1 III–19–26 +
F–12
1st superinfection VII–6–26 +
J–11
D–10
2d superinfection IX–29–26 –
XII–2–26, received 0.090 gram neosalvarsan
B–5
1st reinoculation IV–4–27 –

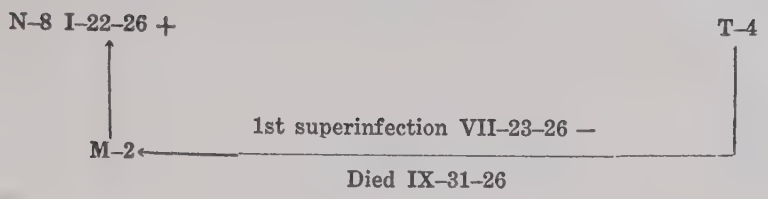

F–12 VII–6–26 +
D–10
1st superinfection VIII–20–26 +
J–13
II–26–27, received 0.08 gram neosalvarsan
B–5
1st reinoculation III–8–27 —

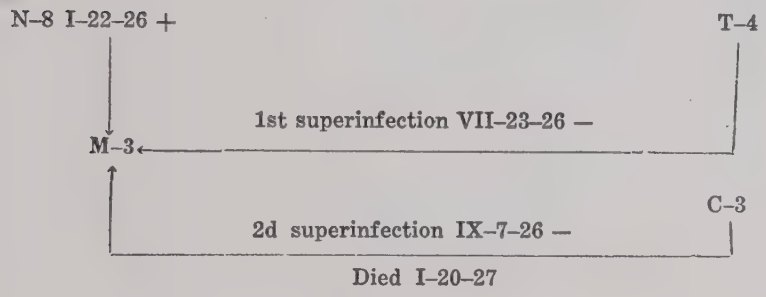

P–8 XII–11–25 +
N–8
1st superinfection III–19–26 +
K–2
F–12
2d superinfection V–24–26 +
F–12
3d superinfection VII–6–26 —
D–10
4th superinfection IX–29–26 —
Died I–3–27

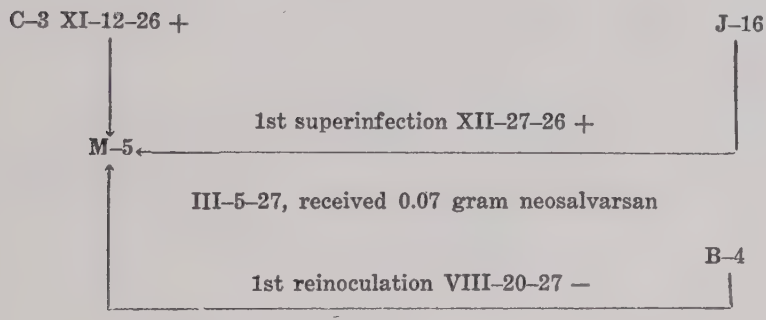

N–8 III–19–26 +
F–12
1st superinfection V–24–26 +
K–3
Died VII–8–26

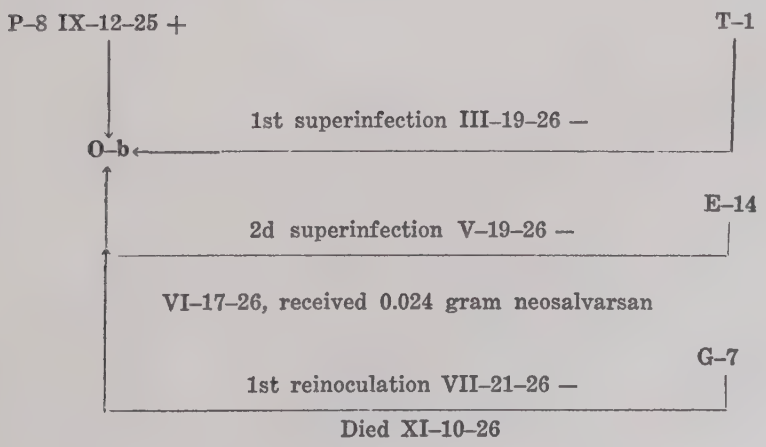

A–3 III–26–25 +
N–8
1st superinfection III–19–26 —
L–4
E–14
2d superinfection V–14–26 —

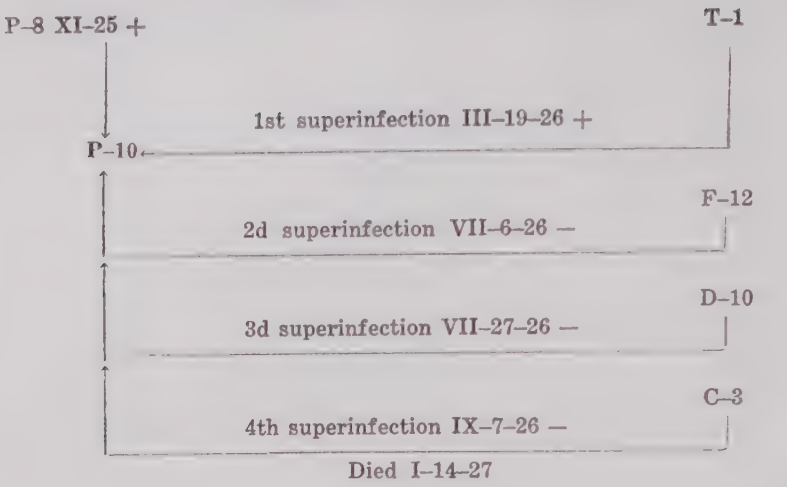

A–3 III–26–25 +—Continued
L–4—Continued
VI–17–26, received 0.024 gram neosalvarsan
G–7
1st reinoculation VII–21–26 —
IX–27–26, received 0.0285 gram neosalvarsan
B–4
2d reinoculation XI–8–26 —
Died I–3–27
J–11 VII–16–26 +
D–10
1st superinfection IX–29–26 +
L–5
XII–2–26, received 0.090 gram neosalvarsan
B–5
1st reinoculation III–8–27 —
B–4 XI–15–26 +
J–16
1st superinoculation XII–27–26 +
L–6
III–5–27, received 0.07 gram neosalvarsan
B–4
1st reinoculation VII–20–27 —
N–8 III–19–26 +
F–12
1st superinfection VII–6–26 +
M
X–1–26, received 0.0240 gram neosalvarsan
D–10
1st reinoculation XI–18–26 —
Died XII–27–26

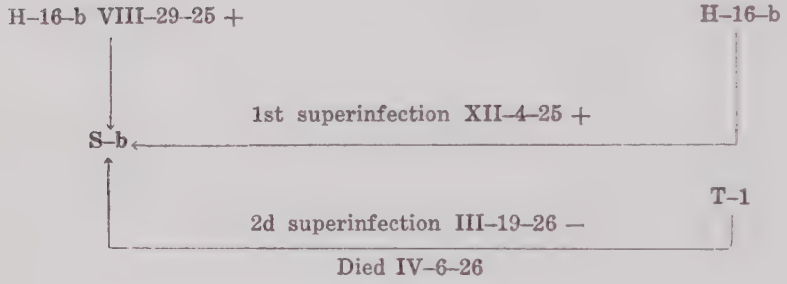

N–8 I–22–26 +
T–4
1st superinfection VII–23–26 —
M–2
Died IX–31–26

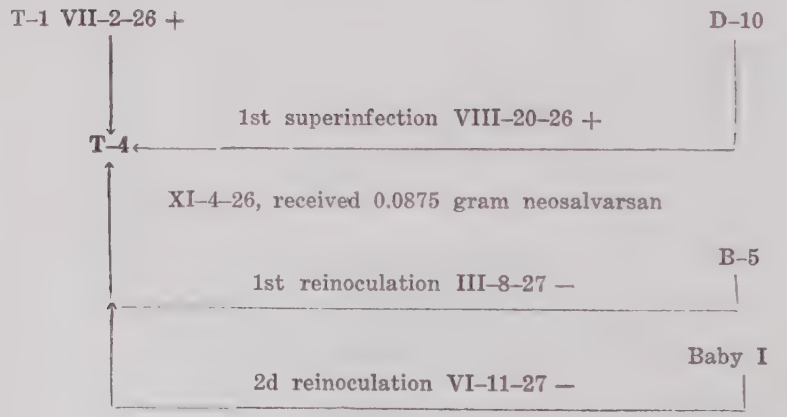

N–8 I–22–26 +
T–4
1st superinfection VII–23–26 —
M–3
C–3
2d superinfection IX–7–26 —
Died I–20–27


C–3 XI–12–26 +
J–16
1st superinfection XII–27–26 +
M–5
III–5–27, received 0.07 gram neosalvarsan
B–4
1st reinoculation VIII–20–27 —

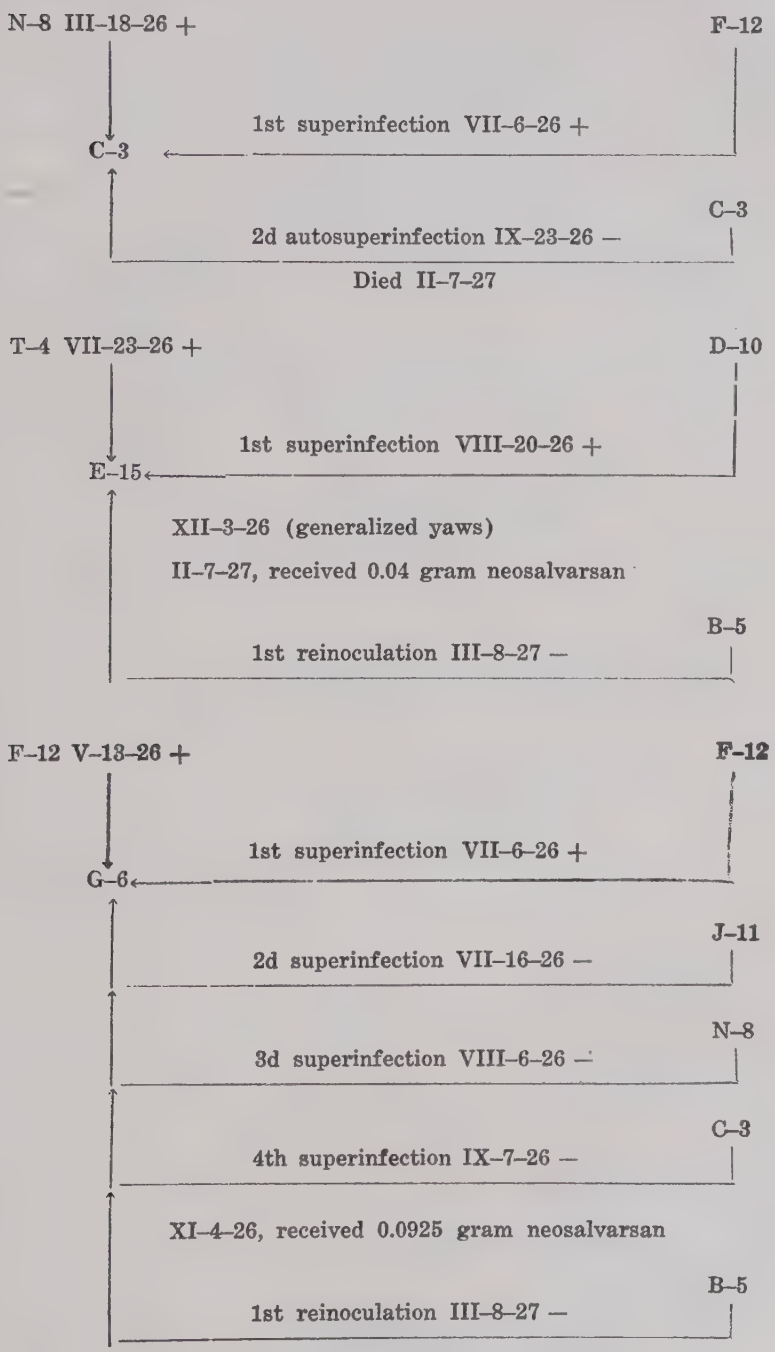

P–8 IX–12–25 +
T–1
1st superinfection III–19–26 —
O–b
E–14
2d superinfection V–19–26 —
VI–17–26, received 0.024 gram neosalvarsan
G–7
1st reinoculation VII–21–26 —
Died XI–10–26

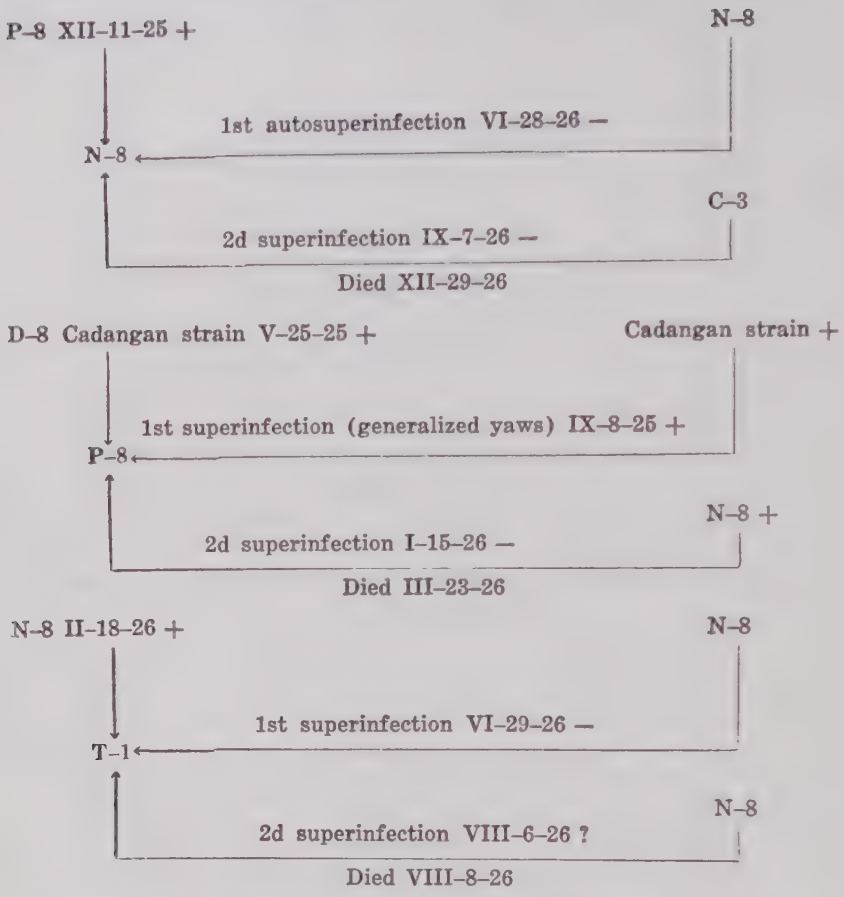

P–8 XI–25 +
T–1
1st superinfection III–19–26 +
P–10
F–12
2d superinfection VII–6–26 –
D–10
3d superinfection VII–27–26 –
C–3
4th superinfection IX–7–26 –
Died I–14–27


H–16–b VIII–29–25 +
H–16–b
1st superinfection XII–4–25 +
S–b
T–1
2d superinfection III–19–26 –
Died IV–6–26

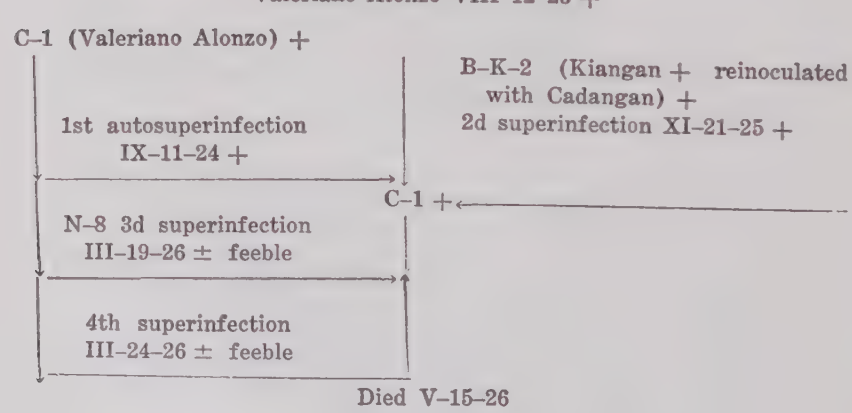

T–1 VII–2–26 +
D–10
1st superinfection VIII–20–26 +
T–4
XI–4–26, received 0.0875 gram neosalvarsan
B–5
1st reinoculation III–8–27 –
Baby I
2d reinoculation VI–11–27 –

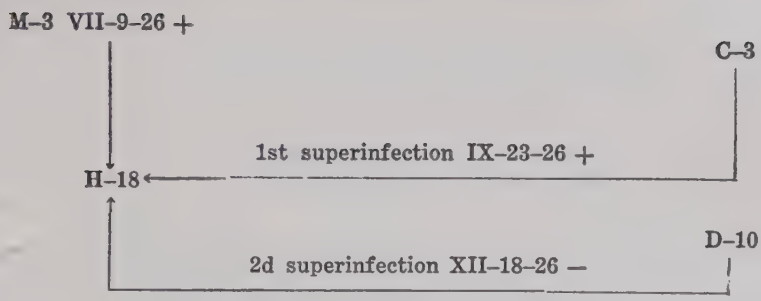
GROUP II. SUPERINFECTION OF POSITIVE YAWS MONKEYS (GENERALIZED YAWS)

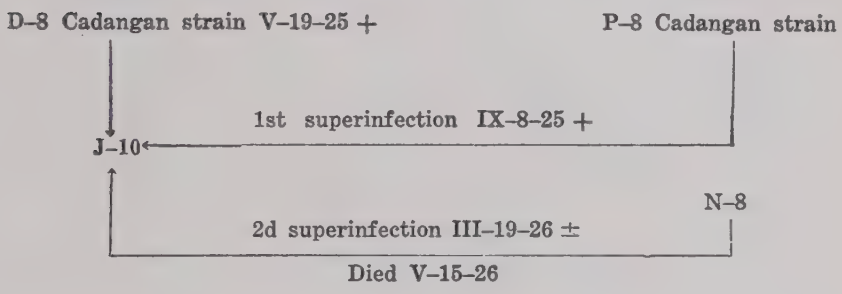




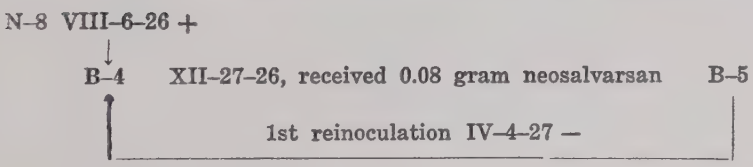
GROUP III. SUPERINFECTION OF POSITIVE YAWS MONKEYS (LATE ULCERATIVE FORM)

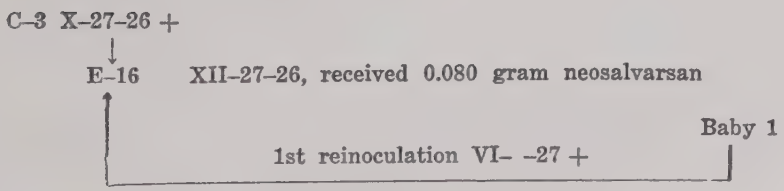


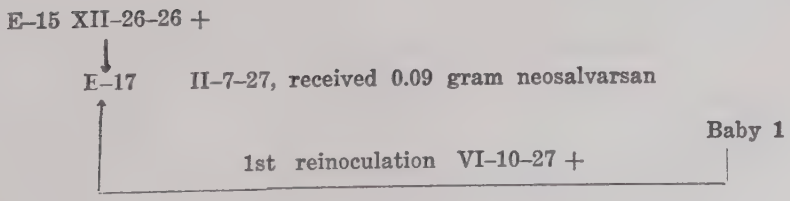


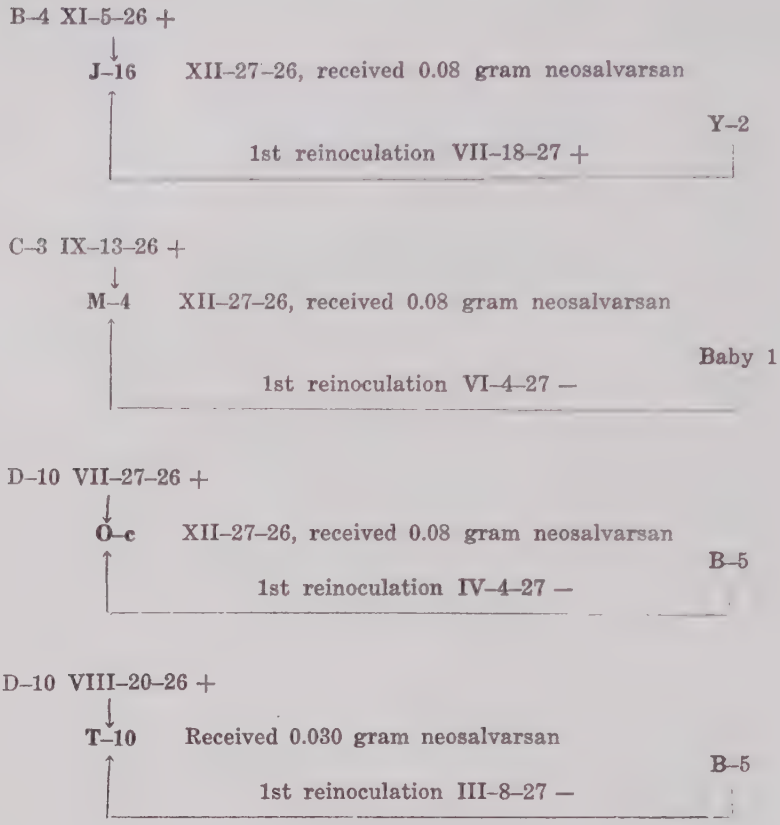


GROUP IV. REINOCULATION OF POSITIVE YAWS MONKEYS








B–4 XI–5–26 +
J–16
XII–27–26, received 0.08 gram neosalvarsan
Y–2
1st reinoculation VII–18–27 +
C–3 IX–13–26 +
M–4
XII–27–26, received 0.08 gram neosalvarsan
Baby 1
1st reinoculation VI–4–27 –
D–10 VII–27–26 +
O–c
XII–27–26, received 0.08 gram neosalvarsan
B–5
1st reinoculation IV–4–27 –
D–10 VIII–20–26 +
T–10
Received 0.030 gram neosalvarsan
B–5
1st reinoculation III–8–27 –

ILLUSTRATIONS

PLATE 1

FIG. 1. The initial lesion of yaws on the eyebrows. Three acuminated papules on the inner canthus and a flat extensive papule on the outer canthus of the eyebrow.

2. A well-developed experimental yaw on the left eyebrow spreading down the bridge of the nose and over the forehead.

PLATE 2

FIG. 1. Initial lesion of experimental yaw on the scrotum. A single papule on the left side of the scrotum and one showing extensive œdema on the prepuce.

2. A well-developed experimental yaw on the skin of the scrotum of a hermaphrodite.

PLATE 3

Extensive local exacerbation on the eyebrows and nose and on the scrotum of a monkey. The original lesion can be seen as a thin line of scabs on the face and on the scrotum. The extensive exacerbation took place within the area already affected by yaws.

PLATE 4

The same monkey as shown in Plate 3, after healing took place and intersected the extensive lesion in such a way that it makes a wrong impression of multiple lesions.

PLATE 5

FIG. 1. Extensive simultaneous lesion over the eyebrows and the nose as a consequence of superinfection, and a forerunner to a general eruption of yaws.

2. Lymphogenic metastases on the left jaw as a consequence of local exacerbation on the eyebrows.

PLATE 6

FIG. 1. Rather dry metastatic yaw over the right elbow.

2. Typical metastatic yaw located above the left ankle.

PLATE 7

Metastatic ringworm yaw on the calf of the left leg of the monkey, showing silvery scale-topped papules arranged in a semicircle. A single papule is visible, about 1 centimeter above the outer ankle.

PLATE 8

Metastatic frambœside in the plica cubiti.

PLATE 9

FIG. 1. Metastatic typical yaws symmetric on the dorsum of the hind feet of a monkey.
2. Metastatic typical yaw on the tail of a monkey.

PLATE 10

Juxta articular location of metastatic lesions on the elbow and wrist; on the left palm, psoriasis palmaris.

PLATE 11

FIG. 1. Juxta articular and symmetric distribution of a typical yaw on the left olecranon and a semicircularly arranged frambœside round the right elbow.
2. Initial local yaw on the nose.

PLATE 12

Experimental psoriasis palmaris frambœsica in a monkey that developed generalized yaws.

PLATE 13

Maplike arranged desquamative frambœside on the abdomen and the inner portion of the thighs, which becomes confluent in some places and simulates seborrhœa.

PLATE 14

Ichthyotic desquamative dermatosis of the tail and branny desquamative frambœside on the inner surface of the right thigh. Below the point where the tail crosses the left leg a pigmented spot is visible, which is all that is left of the metastatic lesion shown in Plate 6, fig. 2.

PLATE 15

The manner in which the primary yaws lesion enters the nostrils and eventually leads to a nasal form of gangosa.

PLATE 16

FIG. 1. A metastatic yaw over the left trochanter, showing the manner of circular spreading while the original yaw becomes stationary.
2. The remnant of a lesion due to superinoculation on the right eyebrow. One of the metastatic lesions that developed as a consequence thereof is located in the nasolabial sulcus.

PLATE 17

FIG. 1. A deep ulcerative lesion on the left ala nasi at the point of superinoculation. Leucoderma and hyperpigmentation over the eyebrows.
2. Spreading primary lesion over the nose from the eyebrows.

PLATE 18

FIG. 1. A beginning ulcerative lesion of the skin at the root of the nose, September 22, 1926.
2. Leaving a deep scar on healing, lesion has involved the soft parts of the nose, November 17, 1926.

PLATE 19

Further development of nasocutaneous form of gangosa in the same monkey as shown in Plate 18.

FIG. 1. December 3, 1926.
2. January 2, 1927.

PLATE 20

FIG. 1. Well-developed gangosa. Same monkey as shown in Plates 18 and 19.
2. Same monkey as shown in fig. 1. The lesion is beginning to heal. Photograph taken one year later than the one shown in Plate 18, fig. 1.

PLATE 21

FIG. 1. Beginning caries of upper incisors as a consequence of gangosa. Same animal as shown in Plates 18, 19, and 20.
2. Multiple metastatic yaws lesions in the face of an experimental monkey.

PLATE 22

FIG. 1. Lupuslike scab forming lesions over the eyebrows. The primary lesion has entered the nose and healed in the skin but, following superinoculation on the right eyebrow, an ulcerative process developed on the septum of the nose. The septum was completely perforated when this photograph was taken.
2. The same monkey as shown in fig. 1. The ulcerative process is beginning to consume the ala nasi. Having entered the nose from the skin, it is destroying the facial part of the nose from inside out.

PLATE 23

FIG. 1. Well-developed case of the nasal form of gangosa.
2. Well-developed case of the nasocutaneous form of gangosa and keratoderma plantare in an experimental monkey.

PLATE 24

FIG. 1. Another case of the nasocutaneous form of gangosa.
2. Keratoderma plantare, the "moth-eaten skin" of the feet of the experimental monkey extending as ichthyotic dermatosis on the dorsum pedis.

PLATE 25

FIG. 1. A case of extensive gangosa of the nasocutaneous form.
2. Anatomical specimen of the case of gangosa in an experimental monkey, showing the excessive granulations and the swelling of the interior parts of the nose.

PLATE 26

Same case as Plate 25, fig. 1, after one injection of neosalvarsan.

PLATE 27

FIG. 1. A swelling between the eyebrows and on the right side of the nose, showing the character of immune reaction in yaws. The general marasmus and alopecia are also shown.

2. Specific swelling of the axillary glands and the right mamillary lymph gland in a case of generalized yaws.

PLATE 28

FIG. 1. Section through an initial lesion of experimental yaw in the skin, showing the papule, and the accumulation of cellular infiltrate within the epidermis.

2. The type of cellular infiltrate in a well-developed yaws lesion surrounding a small blood vessel which remained normal.

PLATE 29

FIG. 1. Section through gangosa, showing acanthosis and the presence of pigment cells in granulation tissue.

2. Section through turbinate of a case of gangosa, showing the superficial manner of spreading of the frambœsic process on the mucous membrane.

PLATE 30

FIG. 1. *Treponema pertenue* in the intestine of a house fly, five hours after feeding on a frambœsic lesion.

2. Section through keratoderma plantare, showing acanthosis, hypertrophy of the epidermis, and keratosis. The subepidermal portion shows an accumulation of pigment cells but very little inflammatory reaction.

TEXT FIGURE

FIG. 1. Diagram showing the results of superinfection and the continuous passage through monkeys of the Juan Cadangan strain of yaws. An asterisk indicates passage through monkeys without superinfection.

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PLATE 1.

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PLATE 2.

PLATE 3.

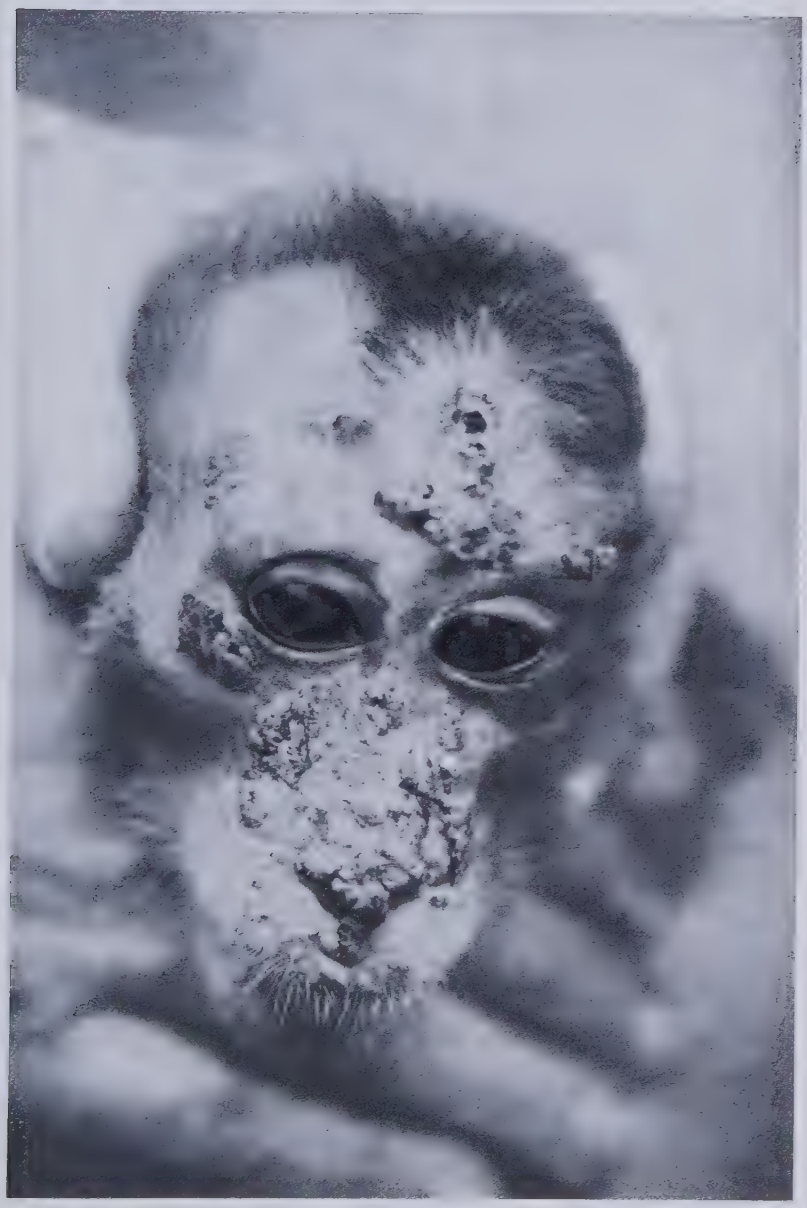

PLATE 4.

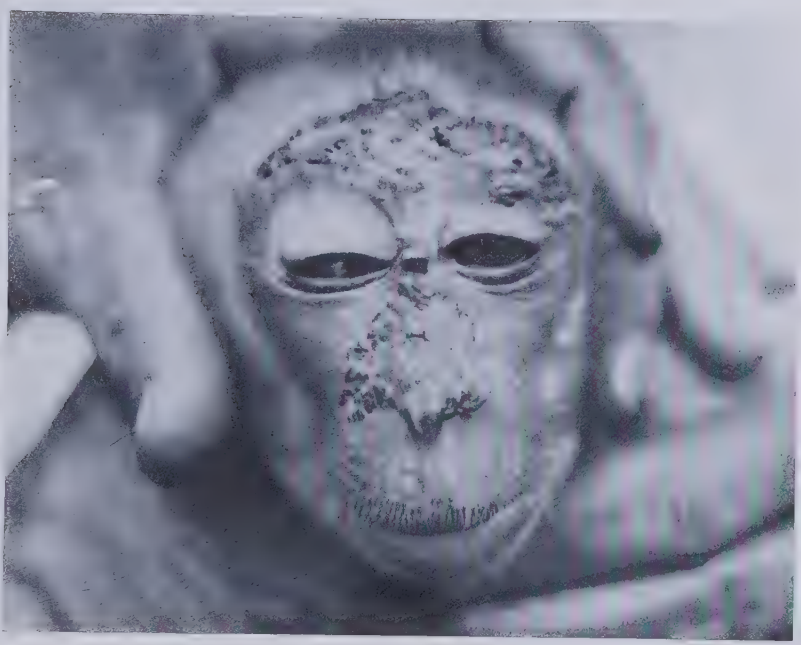

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PLATE 5.

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PLATE 6.

PLATE 7.

PLATE 8.

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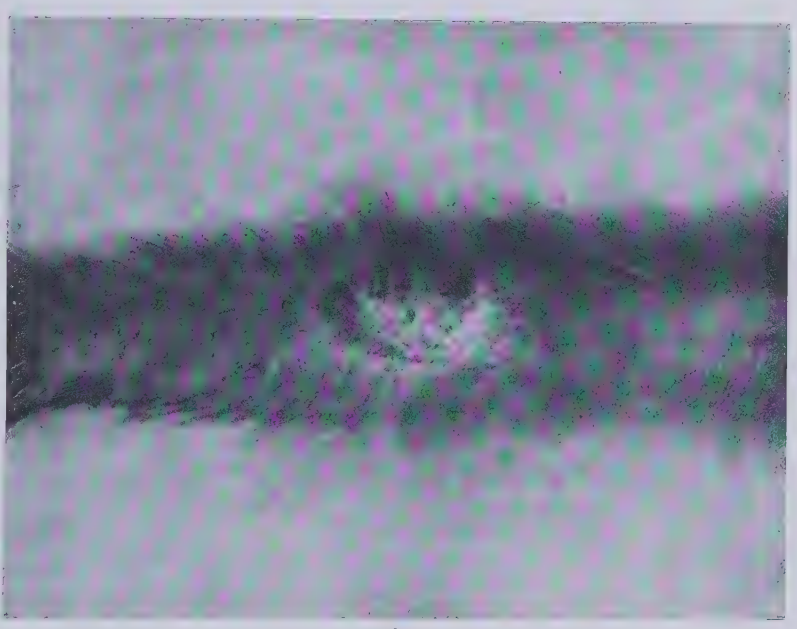

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PLATE 9.

PLATE 10.

1

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PLATE 11.

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PLATE 12.

PLATE 13.

PLATE 14.

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PLATE 15.

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PLATE 16.

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PLATE 17.

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PLATE 18.

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PLATE 19.

PLATE 2

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PLATE 21.

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PLATE 22.

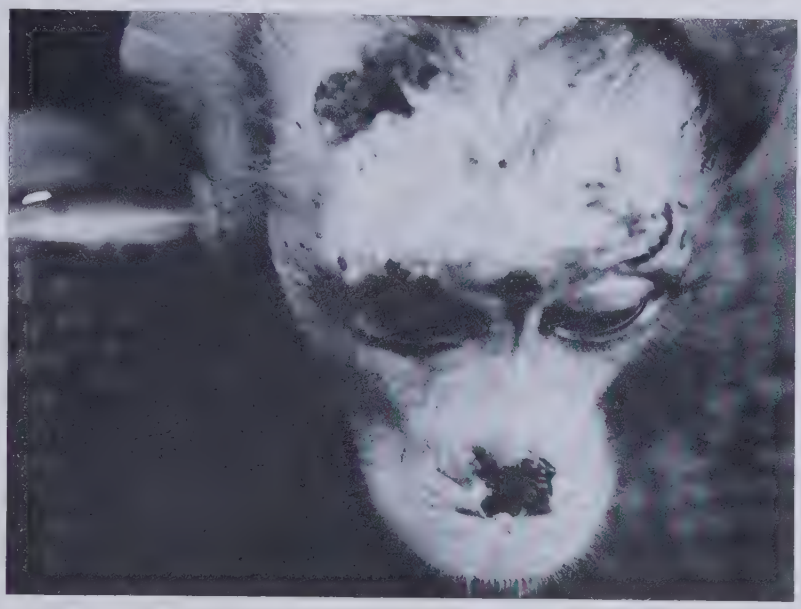

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PLATE 23.

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PLATE 24.

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PLATE 25.

PLATE 26.

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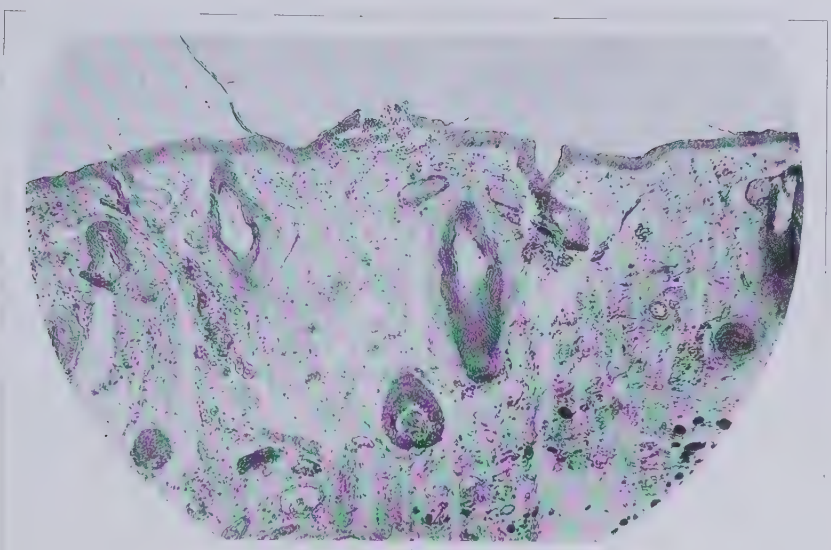
PLATE 27.



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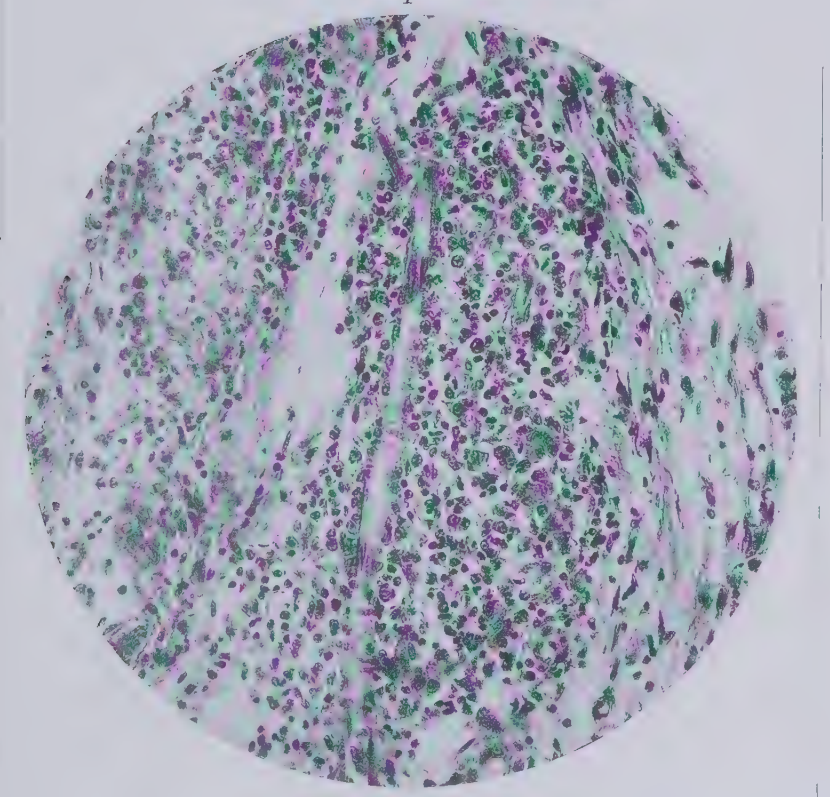

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PLATE 28.

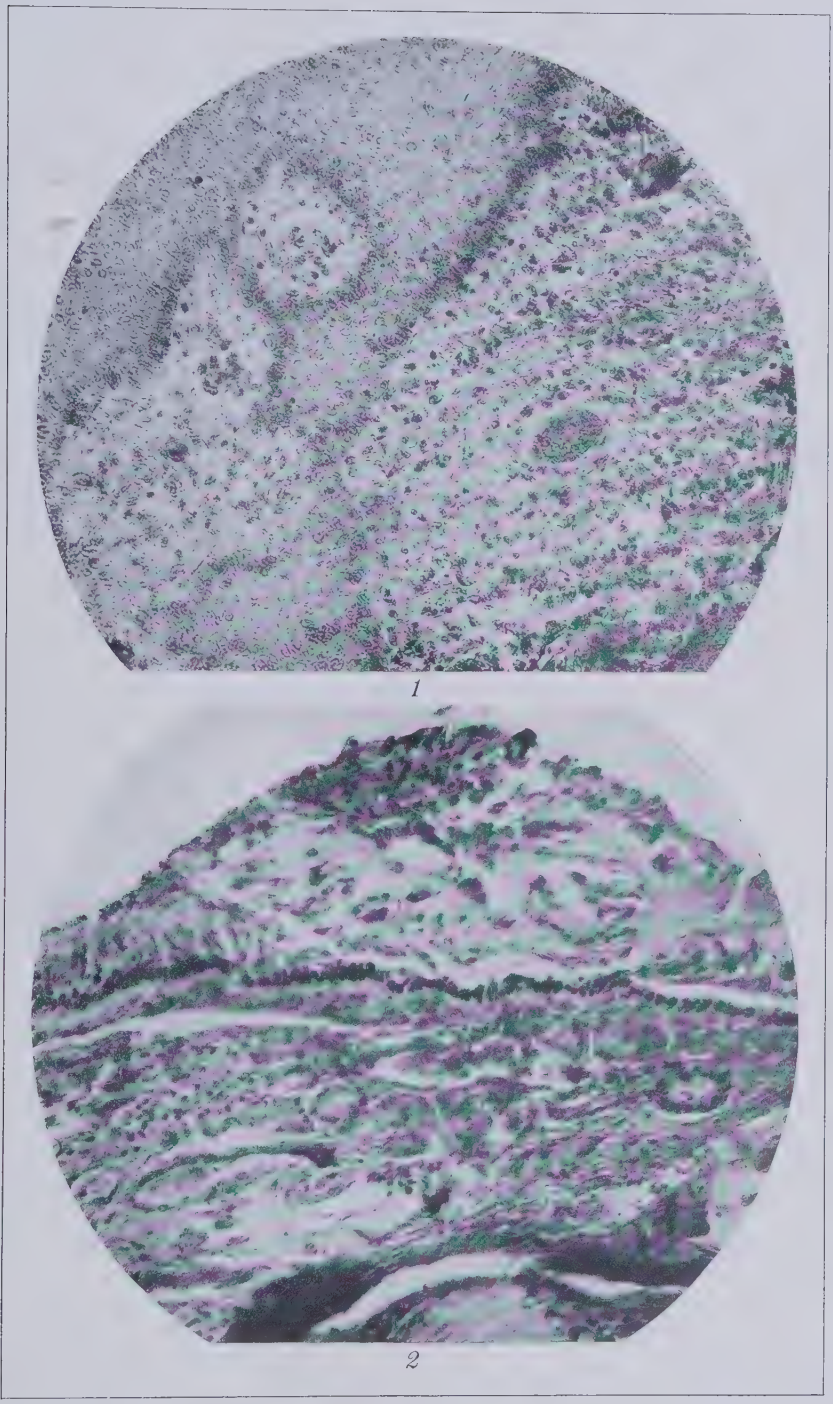

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PLATE 29.

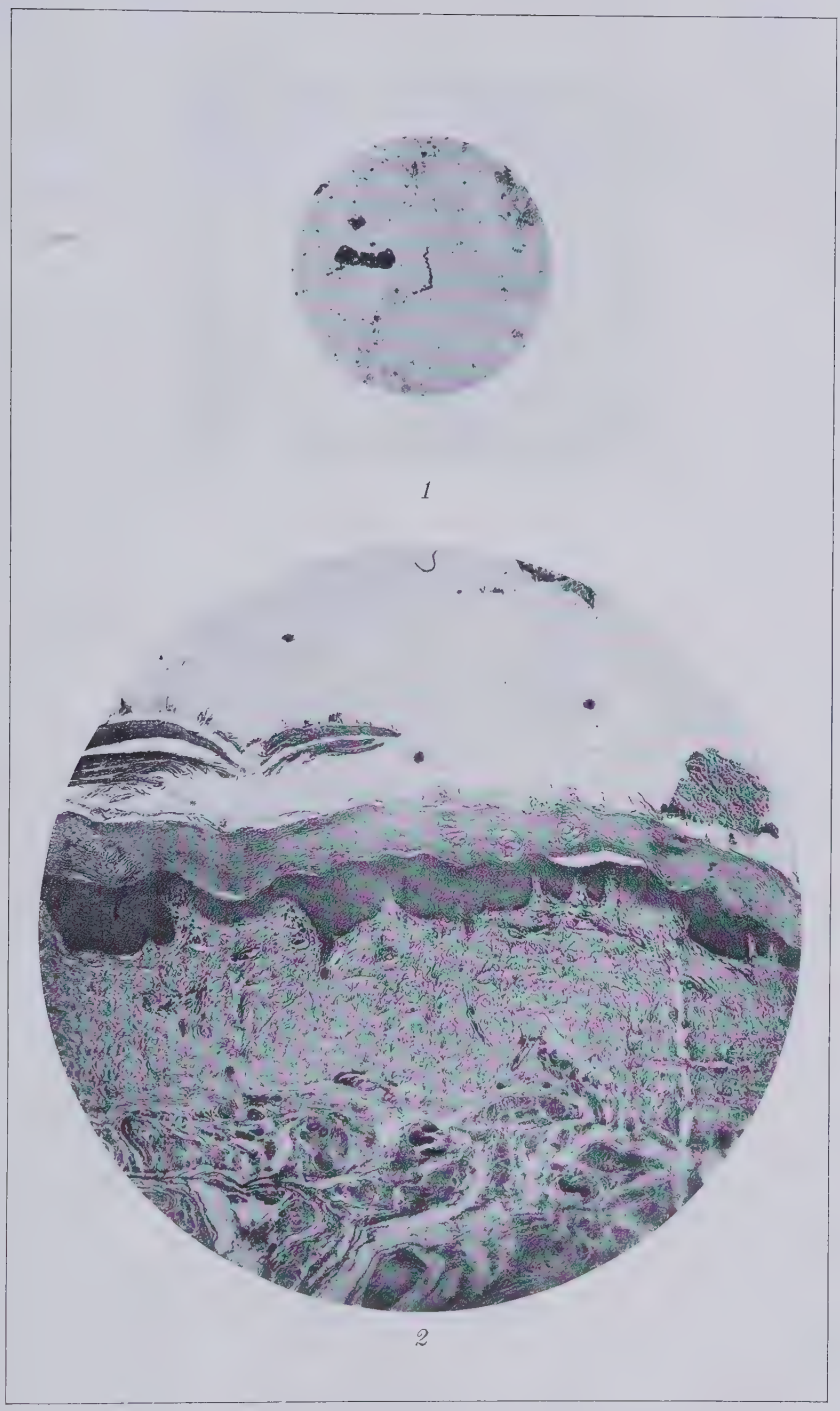

PLATE 30.

VIABILITY OF TREPONEMA PERTENUE OUTSIDE OF THE BODY AND ITS SIGNIFICANCE IN THE TRANSMISSION OF YAWS

By KODO YASUYAMA [1]

Of the Division of Biology and Serum Laboratory
Bureau of Science, Manila

Up to the present and as far as the available references show, very little experimental work on the viability of either *Treponema pertenue* or *Treponema pallidum* has been done. There are fundamental differences between frambœsia and syphilis, as far as the possibilities of transmission are concerned. These differences become apparent from the following considerations:

1. Syphilis is a sexual disease, but frambœsia rarely becomes such and only secondarily.

2. The places of predilection of the primary lesion in syphilis are the mucous membranes; in frambœsia the place of predilection is the skin.

3. Syphilis is a disease that is acquired in the majority of cases after the age of puberty or it is inherited; but frambœsia is not hereditary and is contracted, in the great majority of cases and in endemic localities, before the age of puberty.

It becomes at once evident that the mode of transmission is not likely to be so uniform in yaws as in syphilis and, consequently, the study of viability of *Treponema pertenue* is of great importance in the epidemiology of this disease, and the result of such study may serve as a basis for the rational interpretation of the transmission of yaws.

In order to obtain some data with regard to the possible carriers of *Treponema pertenue* responsible for the transmission of the disease the experiments on viability of *Treponema pertenue* outside the body organism were extended to the study of its viability in or on the bodies of insects, some of which have been incriminated, and others might be, in the transmission of the disease.

As early as 1907 Ashburn and Craig(1) found active *Treponema* in yaws material kept in capillary tubes "for a period

[1] Lieutenant Surgeon, Imperial Japanese Navy.

of several days." There is no indication found in their paper, however, that further experiments were performed by them on the viability of *Treponema pertenue*. Furthermore, I was unable to find any other reference on the subject in the available literature.

EXPERIMENTS ON VIABILITY OF TREPONEMA PERTENUE OUTSIDE OF THE BODY ORGANISM

The experiments here reported were performed with yaws material suspended in various liquids and exposed to room temperature (average 28.5° C.) for varying periods of time. The material was obtained from yaws lesions of experimental monkeys that had been infected with the strain Candangan for other purposes.

MICROSCOPIC EXAMINATION FOR MOTILE ACTIVITY OF TREPONEMA PERTENUE

The typical yaws lesion in monkey was scraped with a sterilized scalpel and the oozing serum, which contained a fair number of *Treponema pertenue*, was used. The microscopical examination was performed by means of dark-field illumination.

Several perfectly clean slides were prepared and a drop of physiological salt solution, or of normal horse serum, or of the serum of the experimental animal, was placed on each slide.

The yaws material was mixed with the drop of the medium on each slide by means of a scalpel; it was then covered with a cover glass and sealed by means of vaseline. Each slide was examined immediately after the sealing under dark-field illumination and only the slides that contained a fair number of active treponemas were kept for further observation.

DISCUSSION OF RESULTS

The motile activity of *Treponema pertenue* is not constant, not even in fresh material from the yaws lesion. Generally speaking, *Treponema pertenue* in the oozing serum from the early lesion is very active, but the treponemas found in an old and healing lesion are usually sluggish or nonmotile.

Although I failed by this arrangement of the investigation to determine the critical point of time when the motility of *Treponema pertenue* outside of the animal's body ceased, the length of time varying considerably in each experiment, yet the results give an indication of useful information as far as this problem is concerned. The initial motile activity of *Treponema pertenue* outside of the living body does not persist longer than three and

a half hours at room temperature. The medium in which the treponemas are suspended appears to be immaterial; that is, no striking difference was noticed whether physiological salt solution, horse serum, or the serum of the experimental animal was used.

However active the treponemas may be in the beginning of the experiment, the movement becomes sluggish within three and a half hours or less. The complete cessation of movement took place within not more than twenty-four hours and in not less than thirty minutes. The appearance of granules in the treponemas takes place after the movement has ceased. This seems to be the beginning of the morphological change in the microorganism. The complete dissolution of the organism did not occur at times even after five days' exposure.

I am fully aware of the fact that the values as to the length of time during which *Treponema pertenue* can be detected or its motility noted outside of the body are not absolute and under various conditions they could probably not be duplicated, owing to the multiplicity of factors involved; but there is a strong indication that the treponemas, judging by their motility, survive but a comparatively short time.

In order to find out how far the immobilization of the treponemas can be taken as an indication of their death, a series of animal experiments was arranged in which the development or nondevelopment of the specific lesion following inoculation was used as an indicator of the viability of *Treponema pertenue*.

TECHNIC

Yaws material suspended in salt solution.—Several sterilized Petri dishes were prepared, each containing 0.5 cubic centimeter of sterilized physiological salt solution. The tissue of the lesion was scraped off with sterilized scalpel and emulsified in salt solution. Dark-field examination of this suspension was repeated and specimens containing treponemas were allowed to stand at room temperature. The suspension was injected into the experimental animal without further dilution at the end of definite periods of time.

The animal's own serum.—A number of sterilized capillary tubes were prepared. The oozing serum was collected into the capillary tubes. Both ends of the capillary tube were then sealed in the gas flame. Care was taken not to heat the material in the tube while sealing the tubes in the flame. The exposure of treponemas suspended in the lesion's own fluid was

made to approach natural conditions as nearly as possible. At the same time, sealing the tubes prevented evaporation and anærobic conditions were thereby achieved.

The capillary tubes were exposed to room temperature (25 to 28° C.), incubated at 37° C., or refrigerated at 0° C. for various periods of time. At the end of the exposure, just before the inoculation was made, both ends of the sealed capillary tubes were broken off and, by shaking gently, the content of the capillary tubes was emptied into sterilized Petri dishes containing, each, 0.5 cubic centimeter of physiological salt solution.

Series of experiments were performed as shown in Table 1. The temperature and the time of exposure of the material containing *Treponema pertenue* are recorded in the table.

TABLE 1.—*Showing the results of the test for viability of Treponema pertenue outside of body organism.*

[Experiment in vivo.]

Length of exposure to various temperatures.	Temperature to which exposed.	Suspended media of treponema.	Date of inoculation.	Result.	Date of appearance of lesion.	*Treponema pertenue* in the lesion.
	°C.					
Immediately	28	Salt solution	VII–9–26	+	IX–20–26	+
30 minutes	28	do	do	+	do	+
60 minutes	28	do	do	—		
2 hours	28	do	do	—		
19 hours	28	do	VIII–10–26	—		
22½ hours	28	do	do	—		
24 hours	28	do	do	—		
30 minutes	28	do	III–24–26	+	V–13–26	+
45 minutes	28	do	do	—		
2 hours	28	do	do	—		
30 minutes	5	do	IX–29–26	—		
Do	37	do	do	—		
1 hour	5	do	do	—		
Do	37	do	do	+	I–17–27	+
Immediately	28	Own serum	X–27–26	+	XII–14–26	+
30 minutes	28	do	do	+	do	+
1 hour	28	do	do	+	do	+
1½ hours	28	do	do	+	do	+
2 hours	28	do	do	—		
Immediately	28	do	XI–16–26	+	XII–14–26	+
30 minutes	28	do	do	+	do	+
1 hour	28	do	do	+	do	+
1½ hours	28	do	do	+	do	+
Immediately	28	do	XI–26–26	+	I–26–27	+
2 hours	28	do	do	+	do	+
2½ hours	28	do	XI–17–26	—		
4 hours	28	do	do	—		
1½ hours	37	do	I–24–27	+	III–10–27	+
Do	0	do	do	—		

TABLE 1.—*Showing the results of the test for viability of Treponema pertenue outside of body organism*—Continued.

Length of exposure to various temperatures.	Temperature to which exposed.	Suspended media of treponema.	Date of inoculation.	Result.	Date of appearance of lesion.	*Treponema pertenue* in the lesion.
	°C.					
Two hours	37	Own serum	I-24-27	+	III-23-27	+
Do	0	do	do	—		
Thirty minutes	37	do	II-3-27	—		
Do	0	do	do	—		
Six hours	28	Salt solution	XI-29-26	—		
Immediately	28	do	do	+	XII-29-26	+
One hour	37	Own serum	IV-11-27	—		
Do	0	do	do	—		
One and one-half hours	37	do	do	+	VI-1-27	+
Do	0	do	do	—		
Three hours	0	do	IV-12-27	—		
Do	0	do	do	—		
Do	37	do	do	—		
Do	37	do	do	—		
Four hours	37	do	V-12-27	—		
Do	37	do	do	—		
Do	0	do	do	—		
Do	0	do	do	—		
Five hours	37	do	do	—		
Do	37	do	do	—		
Do	0	do	do	—		
Do	0	do	do	—		

Such animals as developed no sign of yaws were kept under observation for at least forty days before they were discarded. The inoculation of yaws material suspended in physiological salt solution and exposed to room temperature for thirty minutes resulted in a positive take. The same suspension of yaws material exposed to room temperature for forty-five minutes, for one hour, for two hours, for six hours, for nineteen hours, for twenty-two and one-half hours, and for twenty-four hours gave no takes.

Inoculation of yaws material kept in the donor's own serum and in sealed capillary tubes, when exposed to room temperature for thirty minutes, for one hour, for one and one-half hours, and for two hours, resulted in the development of yaws. Yaws material exposed for two hours, for two and one-half hours, and for four hours gave no takes.

The inoculation with yaws material suspended in physiological salt solution and exposed to body temperature (37° C.) for thirty minutes and for one hour produced yaws. The same material,

suspended in the same solution but exposed to a temperature of 5° C., gave negative results in both experiments with material exposed for thirty minutes and for one hour.

The experiments with the material suspended in the donor's own serum and kept in capillary tubes, when exposed to body temperature (37° C.) gave positive results after exposure of one hour, one and one-half hours, and two hours. Negative results were obtained in monkeys inoculated with the same material incubated at 37° C. when exposed for four hours and for five hours.

Inoculation of yaws material suspended in the donor's own serum and exposed to 0° C. for thirty minutes or more gave negative results without exception. The yaws material was kept in an electric refrigerator for thirty minutes, for one hour, for one and one-half hours, for two hours, for three hours, for four hours, and for five hours. From these experiments the conclusion can be drawn that *Treponema pertenue* remains viable at freezing point for less than thirty minutes.

The fact that *Treponema pertenue* does not survive at low temperature but, as shown in our experiment, dies in less than half an hour, I propose to offer as one (probably the main) factor responsible for the well-known fact that frambœsia tropica does not gain a foothold when introduced into a temperate climate. Cases are on record of frambœsia acquired by inhabitants of a moderate climate in the Tropics. Upon return of the patient from the Tropics to his homeland in a temperate climate, the disease ran its natural course, but no subsequent cases developed in his community.

Naturally, the factor given above is not the only one, and the different condition of the skin in the Tropics, due to high temperature and excessive humidity, from that encountered in the temperate climate, is another important factor that might also be responsible for the prevalence of yaws in hot countries and the nonexistence of frambœsia in cold countries. The modification of the clinical manifestation of yaws at high altitudes in the tropical Philippines has been brought out by Lopez-Rizal and Sellards.(8)

THE VIABILITY OF TREPONEMA PERTENUE IN AND ON INSECTS

Some of the fundamental points with regard to the viability of *Treponema pertenue* outside of the body organism having been ascertained, I extended my experiments to the problem of its viability in and on insects, in order to find some clue as to the

particular insect most likely to be responsible for the transmission of yaws. That the direct transmission of frambœsia from person to person is undoubtedly one of the ways of spreading and maintaining the disease has been conclusively demonstrated by experimental transmission of yaws from patient to animal or to man. That there is, however, more than one mode of transmission and that insects are the transmitters of the disease, has been generally suspected by workers in many tropical countries. Of all these insects, the fly has been most frequently incriminated, owing to its habits of feeding on secreting and oozing skin lesions.

According to Gudger,(4) the earliest reference as to the transmission of yaws bears date of 1769 from Guiana. The second, while of much later date (1817), indicates that in Brazil at that time the infection was conveyed by a certain fly recognizable by its small size. Walsh(14) published in 1831 an article in which he stated that the eye sometimes is partially affected and a small fly is then attracted by the discharge; this insect comes loaded with the contagious matter and communicates it to the next person on whose face it happens to light. Stedman(13) wrote in his description of the expedition to South America in 1796 as follows:

> The yaws, * * * if a fly which has been feeding upon the disease (and they are generally covered with them) light upon the slightest scratch on a healthy person, it communicates this dreadful disorder, which always confines him for several months.

Kester(7) in 1817 described the transmission of yaws as follows:

> This horrible disorder (the yaws) was contracted by inhabiting the same room with the patient, and by inoculation; this is effected by means of a small fly from which every precaution is often times of no avail; great numbers of the insects of this species appear in the morning, but they are not so much seen when the sun is powerful; if one of them chances to settle upon the corner of the eye or mouth, or upon the most trifling scratch, it is enough to inoculate the bobas, if the insect comes from a person who labors under the disease.

Robertson(11) in 1908 reported that he observed that a large number of flies infested the houses of natives suffering from yaws, and that they were in frequent contact with the prevalent secretions of the papules on the bodies of the inmates. In his second report (1910) Gudger(5) makes the following statement:

> The yaws are * * *, and a small quantity of yellowish pus is usually seen adhering to their surface, which is commonly covered with flies, * * * it is usually believed that this disorder is communicated

by the flies which have been feasting on a diseased object, to those persons who have sores, or scratches, which are uncovered.

In 1911, Howard(6) stated that there is strong circumstantial evidence that tuberculosis, anthrax, yaws, etc., might be and were so carried. Castellani and Chalmers(3) in 1919 wrote as follows:

In our opinion there can be little doubt that in certain cases insects may carry the disease. It is very noticeable that flies eagerly crowd on the open sores of frambœsia patients.

Oho(10) in 1921 reported as follows:

In my investigation I was further able to ascertain that innumerable flies were sitting on the moist surfaces of the framboesic eruptions and that they sucked the secretion * * *; one finds in the habitations mostly *Musca domestica* and outdoors a yellow kind of fly *Musca xanthomera* (?). The last mentioned sucks with more zeal on small wounds, such as scratches and thereby produces pain, so that the primitives, who have for instance a small wound on the foot, cannot remain still (on account of the pain caused by the flies). This fly is possibly mostly to be blamed for the transmission of the infection.[2]

Some authors mention insects other than flies as responsible for the dissemination of yaws. Bahr(2) has suggested that the causative treponemas may be transmitted by some blood-sucking insect the range of which is definitely limited by the character of the vegetation or by climatic factors. Modder and Glas(9) concluded, from their yaws-inoculation experiments on birds, that the possibility of transmission of yaws by means of the argas and the ixodes varieties of tick could be easily demonstrated by any one who would try the experiment, because of the limitation of yaws to certain areas in Ceylon where these ticks are found. In the same paper the incubation period is given as six days. It is beyond our belief that a yaws lesion can be produced after so short an incubation period and that fowls are susceptible to frambœsia. There is, however, at present no experimental evidence to the contrary. Castellani and Chalmers(3) state that "Ants also are occasionally seen to go on to the frambœtic ulcerations, as well as on to ordinary ulcers."

From the above-quoted references it is impossible to form a definite opinion as to whether or not insects are involved in the transmission of yaws and, if they are, which insects are responsible for the transmission.

[2] This quotation has been translated by Dr. O. Schöbl.

It is generally known that yaws is a household disease; that is, members of the same household are commonly infected. Furthermore, endemic villages are not unusual in the Philippine Islands.

The question of prevalence of yaws in the lowlands and its comparative rarity or absence in high mountains has been frequently discussed in the literature. A definite line of elevation has been drawn at 800 feet which has supposedly never been crossed by yaws. Oho,(10) however, in Formosa, and Lopez-Rizal and Sellards(8) in the Philippines, have demonstrated that frambœsia can be found at much higher altitudes than is generally believed. This geographic and climatic selectivity of yaws has been taken by Bahr(2) as an indication that yaws is transmitted by special insects of similar geographic and climatic distribution. This explanation apparently does not consider the factor of viability of the causative agent, which was brought out in my researches. With regard to transmission, an experimental fact and a clinical observation must be considered. Lopez-Rizal and Sellards observed modification of the clinical yaws lesion at high altitudes; briefly stated, the yaws lesions were restricted among patients residing at high altitudes to warm moist folds and body orifices.

In my experiments the fact was brought out that *Treponema pertenue* outside of the body organism survives only a few hours and is so susceptible to low temperature that it becomes inert and can no longer produce a lesion when exposed to freezing temperature for even a few minutes, while at blood temperature it survives longer than at room temperature (average 28.5° C.). If the shortness of life of *Treponema pertenue* at low temperature be taken into consideration on one hand, and the modification of clinical yaws by low temperature at high altitude due to the condition of the skin on the other, it at once becomes evident that no particular insect restricted to this geographic distribution need be looked for as a carrier of yaws.

Indeed, common, geographically ubiquitous insects such as flies are more likely to be responsible for the transmission than is an insect of some particular geographic distribution. If the enormous numbers of treponemas discharged at times by the yaws lesions and the persistency with which flies feed on such oozing lesions as yaws be considered, then it becomes clear that a fly, of all insects hitherto accused, is most likely the one

responsible for the transmission of yaws. The disinclination of blood-sucking insects, such as bedbugs and mosquitoes, to feed directly on yaws lesions, and scarcity of *Treponema pertenue* in the circulating blood, must be considered.

EXPERIMENTS ON VIABILITY OF TREPONEMA PERTENUE ON AND IN THE BODY OF INSECTS

Such insects as were suspected to be responsible for the transmission of yaws were used in these experiments. House flies and mosquitoes were kept in special cages of a wooden frame and mosquito-net walls.(12) The flies were fed on bananas and the mosquitoes on sugar water in the cages while awaiting the experiments. Bedbugs were kept in wooden boxes and maintained by feeding them on human blood. Immediately before the feeding experiment the insects were placed in sterilized individual test tubes which were then stoppered with cotton plugs.

The feeding of insects upon the yaws lesion was performed as follows: First of all, the crust was removed from the surface of the lesion and the presence of treponemas in the oozing liquid was ascertained. If the typical oozing did not take place immediately, this was provoked by gentle scratching of the surface of the lesion by means of a sterile scalpel. The lesion having been prepared in this manner, the particular insect, now confined in a sterile test tube, was selected. The test tube containing the insect was turned bottom up. The cotton plug was removed and the test tube was placed quickly over the lesion in such a manner that the mouth of the test tube was placed tightly on the surface of the lesion. By gentle tapping on the bottom of the test tube the insect was brought into direct contact with the surface of the lesion, where it could feed freely on the yaws material.

After the insect had fed it was killed by chloroform vapors or by crushing. The mouth of the test tube was sterilized by means of the gas flame.

The dead insect was placed in a sterilized mortar, crushed, and ground up, and the resulting pulp was suspended in 0.5 cubic centimeter of physiological salt solution.

In some instances the insects, after having been fed on yaws material, were dissected and the intestine alone, which was found to contain treponemas, was emulsified as described above.

The emulsion thus prepared was injected intradermally into normal monkeys.

DESCRIPTION OF PROCEDURE AND TECHNIC OF MICROSCOPIC EXPERIMENTS

The dead insect that had fed on the yaws lesion was removed from the test tube and its legs and wings were removed. The body was then placed in the center of a microscopic slide. The slide was brought under the dissecting microscope and the body was divided by means of needles into three parts; namely, head, thorax, and abdomen. Each of the three parts of the insect's body was placed in a drop of normal saline solution on a separate slide. Then each part (that is, the head, the thorax, and the abdomen) was crushed with needles and the crushed part was transferred to another slide. Thus, two slides were prepared with the material obtained from the insect's head, two with that from the thorax, and two with that from the abdomen. One slide was examined immediately under the dark-field microscope; the other slide was stained by Fontana's method, slightly modified by me.

The results of the microscopic examination are given in Tables 2 to 4. During the feeding, the flies willingly started to take their meal from the lesion. Within three to five seconds the feeding had been accomplished. After this time they could not be induced to take more food from the oozing yaws lesion. It happened very often that mosquitoes, when they were induced to alight on a yaw, adhered to the moist surface of the lesion with their slender legs and consequently perished. They preferred naturally to suck blood on the normal skin through which they could easily introduce their sharp blood-sucking instrument (the proboscis). Accordingly, the mosquitoes that were subjected to the feeding experiment alighted, if at all, on the marginal part of the lesion only, and not on the lesion proper. It took thirty to fifty seconds before the abdomen bulged with blood. Bedbugs likewise refused to approach the dangerous moist surface of the yaws lesion; they sucked blood on the margin of the lesion, where the skin was dry. The feeding experiments with flies were successfully carried out without much difficulty, but those with mosquitoes ended with the result that only twenty-seven of the two hundred mosquitoes that were used in the experiments fed at all, and on the margin of the lesion at that.

TABLE 2.—*Showing the results of microscopical examination of flies fed upon yaws lesions.*

[+, positive finding; —, negative finding; 0, not done.]

Treponema in the lesion of the donor.	Time between feeding and microscopical examination.	Number and kind of flies examined.	Result of microscopic examination.					
			Head.		Thorax.		Abdomen.	
			Dark-field.	Fontana method.	Dark-field.	Fontana method.	Dark-field.	Fontana method.
+	Immediately.	2 house flies	—	—	—	—	++	+
+	do	do	—	—	—	—	++	—
+	do	1 house fly	—	—	—	—	+	0
+	do	1 bluebottle fly indirectly fed.	+	+	+	+	—	—
+	do	1 house fly	+	—	+	—	—	—
+	do	5 house flies	—	—	—	—	—	—
+	do	3 bluebottle flies	—	—	—	—	—	—
	Hrs. min.							
+	0 10	1 house fly	—	—	—	—	—	—
+	0 10	1 bluebottle fly	+	0	—	0	+	—
+	0 30	1 house fly	—	—	—	—	—	—
+	0 40	do	0	0	0	0	+	—
+	1 0	do	—	0	—	0	+	+
+	1 30	2 house flies	—	0	—	0	++	—
+	1 45	1 house fly	—	0	—	0	+	—
+	2 0	do	0	0	0	0	+	—
+	2 0	do	—	0	—	0	—	0
+	2 10	do	—	0	—	0	—	0
+	2 20	do	0	0	0	0	+	+
+	2 30	do	—	0	—	0	—	0
+	2 30	do	—	0	—	0	+	+
+	3 10	do	—	0	—	0	—	0
+	4 15	do	—	0	—	0	+	+
+	4 40	do	—	0	—	0	+	+
+	4 45	do	—	0	—	0	+	—
+	5 50	do	0	0	0	0	+	+
+	5 55	do	0	0	0	0	+	+
+	6 45	do	0	0	0	0	+	—
+	7 0	do	—	0	—	0	—	0
+	7 15	do	0	0	0	0	+	+
+	10 30	2 house flies	0	0	0	0	—	0
+	11 0	1 house fly	0	0	0	0	—	0
+	11 0	do	0	0	0	0	+	+
+	23 0	2 house flies	—	0	—	0	—	0
Total		44 flies						
Positive			3	1	2	1	22	10

TABLE 3.—*Showing the results of microscopical examination of mosquitoes (Stegomyia fasciata) fed upon yaws lesions.*

[+, positive finding; —, negative finding; 0, not done.]

Treponema in the lesion of the donor.	Time between feeding and microscopical examination.	Mosquitoes examined.	Result of microscopic examination.					
			Head.		Thorax.		Abdomen.	
			Dark-field.	Fontana method.	Dark-field.	Fontana method.	Dark-field.	Fontana method.
+	Immediately	4	—	0	—	0	—	0
+	do	3	—	—	—	—	—	—
+	do	3	0	0	0	0	+++	—
+	do	1	0	0	0	0	+	—
	Hrs. *min.*							
+	1 0	3	0	0	0	0	—	0
+	1 30	1	0	0	—	0	—	0
+	1 35	1	0	0	—	0	—	0
+	1 45	1	0	0	—	0	—	0
+	2 0	1	0	0	0	0	—	0
+	2 10	1	0	0	—	0	—	0
+	3 30	1	0	0	—	0	—	0
+	3 45	1	0	0	—	0	—	0
+	4 0	2	0	0	—	0	—	0
+	4 15	2	0	0	—	0	—	0
Total		25						
Positive							4	

TABLE 4.—*Showing the result of microscopical examination of bedbugs fed upon yaws lesions.*

[+, positive finding; —, negative finding; 0, not done.]

Treponema in the lesion of the donor.	Time between feeding and microscopical examination.	Bedbugs examined.	Result of microscopic examination.					
			Head.		Thorax.		Abdomen.	
			Dark-field.	Fontana method.	Dark-field.	Fontana method.	Dark-field.	Fontana method.
+	Immediately	1	—	—	—	—	+	—
+	do	1	—	—	—	—	—	+
+	do	1	—	—	—	—	—	—
+	1 hour	1	+	—	+	—	—	—
+	2 hours	1	+	—	—	—	—	—
Total		5						
Positive			2		1		1	1

The experiment with bedbugs was practically a failure, because only five bedbugs out of more than fifty would feed on the lesion proper.

Textbooks of entomology state that blood-sucking insects such as mosquitoes and bedbugs suck blood by piercing the skin by means of their slender probosci which are provided with sharp tips and enter the capillaries of cutaneous tissue; the sucking action is caused by the blood pressure and by the capillary attraction through the probosci. It is unnatural for blood-sucking insects to feed on oozing blood or on the surface of a skin lesion, because such blood is devoid of pressure.

Flies have wide canals in the proboscis which are distended in the labella. The sucking is accomplished by the negative pressure which is produced by the action of a muscle around the proboscis.

Of the forty-four flies that were satisfactorily fed on the lesion of the donor, three flies contained treponemas in the head, two flies in the thorax, twenty-two flies in the abdomen, and two flies in both parts of head and thorax. Whenever the abdomen of a fly was found bulged with blood, it was possible to demonstrate treponemas microscopically in the intestine of the insect. Treponemas were demonstrated by dark field in the intestine of a housefly as long as eleven hours after feeding.

Of the total number of twenty-five mosquitoes that sucked the blood from the margin of the lesion and the abdomens of which were noted to be bulged with blood, four contained treponemas in the intestine.

Of the five bedbugs which sucked blood on the margin of the lesion of the donor, two contained treponemas in the head, one in the thorax, and one in the abdomen.

The results of our experiments with blood-sucking insects such as mosquitoes and bedbugs show that, if these insects can be induced to feed on the lesion, a high percentage become mechanical carriers of treponemas; but, in as much as observation teaches that these blood-sucking insects show a great disinclination to feed on yaws lesions, they do not enter as a serious factor in the indirect transmission of yaws.

ANIMAL EXPERIMENTS ON VIABILITY OF TREPONEMA PERTENUE IN AND ON INSECTS

It having been found that, of all the insects experimented with, house flies are most likely to be carriers of treponemas

in and on their bodies, the emulsion of the house flies that were fed on the yaws lesions was inoculated into monkeys. The results of these experiments are given in Table 5.

TABLE 5.—*Showing the results of the test for viability of Treponema pertenue in and on insects.*

Insect.	Part of insects used.	Time of exposure.	Treponema in the donor's lesion.	Date of inoculation.	Date of appearance of lesion.	Treponema in the lesion of the receiver.
House fly	Entire body	Immediately	Few	XI–12–26	—	
Do	do	do	do	XI–15–26	—	
Bedbug	do	do	Fair number	do	—	
		Hrs. min.				
House fly	Abdomen	1	Numerous	XI–26–26	—	
6 house flies	Entire body	20	Few	I–11–27	II–28–27+	+
3 house flies	do	20	do	I–14–27	—	
4 house flies	do	15	Fair number	I–15–27	II–28–27+	+
Do	do	15	do	do	do. +	+
13 house flies	Intestine	1	do	II–1–27	—	
5 house flies	Entire body	30	do	II–9–27	IV–2–27+	+

A monkey which received two inoculations with the material exposed in and on the fly's body for twenty minutes, gave a positive and a negative result, respectively. A monkey which received two inoculations with the material exposed on and in the fly for fifteen minutes, gave positive results from both. A monkey which received the material exposed for thirty minutes, gave positive results. From these experiments, it is clear that treponemas survive in and on the body of the house fly for thirty minutes. In other words, *Treponema pertenue* survives on and in the body of the flies about the same length of time as it does in physiological salt solution.

Transmission experiments of yaws to monkeys, by flies fed on the lesion, were performed by Castellani and Chalmers(3) and their coworkers prior to my experiment. The details are given as follows:

> One of us made some experiments to prove that flies are instrumental in the dissemination of the disease. A number of flies were fed on scrapings from slightly ulcerated frambœtic papules. * * * On examination after feeding, the majority presented coarse spirochætes, and a few of them also *T. pertenue*. In another experiment flies fed on yaws material were placed on scarified spots over the eyebrows of several monkeys, and kept there for two hours by means of strips of gauze smeared with collodion at their margins. One of the monkeys became infected.

CONCLUSIONS

1. Up to a certain limit the so-called motility of *Treponema pertenue* can be used as an indication of its viability.

2. The viability of *Treponema pertenue* outside of the body proved to be of short duration.

3. The temperature of the human body (37° C.) proved to be more favorable to the survival of *Treponema pertenue* than did room temperature (average 28.5° C.).

4. At low temperature (0 to 5° C.) the period of viability of *Treponema pertenue* is very limited (less than thirty minutes).

5. The period of viability on and in the body of flies is short.

6. The inability of *Treponema pertenue* to survive at low temperature is given as one of the factors responsible for the fact, that, when introduced into a cold country, yaws will not gain a foothold.

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